2021 NHLBI SYSTEMS BIOLOGY SYMPOSIUM

MAY 12-13, 2021

AN ALL-VIRTUAL EVENT

#SystemsBio2021

NIH MEDICAL ARTS

Table of Contents

Agenda

Agenda

Wednesday, May 12, 2021

9:00-9:07am

Welcome and Opening Remarks
Keji Zhao, Ph.D.
Organizing Committee Chair,
National Heart, Lung and Blood Institute, NIH

Michael M. Gottesman, M.D.
Deputy Director of Intramural Research, Office of Intramural Research, NIH

Session 1 Omics

Moderator: Susan Harbison, Ph.D.

*Speaker Presentations slots are 25 minutes + 5 minutes Q&A + 5 minutes transition time

9:10-9:40am

Nuclear Condensates in Gene Regulation and Disease
Richard Young, Ph.D., Whitehead Institute, Massachusetts Institute of Technology

9:45-10:15am

Epigenetic Basis of Single-Cell Heterogeneity
Keji Zhao, Ph.D., National Heart, Lung, and Blood Institute, NIH

10:15-10:45am

Morning Break

10:45-11:15am

Integrative Approaches Probing Disrupted Cellular Homeostasis in Metabolic Diseases
Anna Greka, M.D., Ph.D., Harvard Medical School, Brigham & Women's Hospital, Broad Institute of MIT

11:20-11:50am

New Advances in Single-Cell 3D Genome Structures and High Precision Transcriptomics
Xiaoliang Sunney Xie, Ph.D., Peking University

11:50am-12:20pm

Pre-Recorded Poster Presentations Segment #1

12:20-1:15pm*

Lunch Break

*12:20-12:50pm

Poster Presenters Break-Out Rooms

Agenda

Wednesday, May 12, 2021

Session 2 Systems Imaging

Moderator: Mark Knepper, M.D., Ph.D.

*Speaker Presentations slots are 25 minutes + 5 minutes Q&A + 5 minutes transition time

1:15-1:45pm	**3D Imaging of Cells by FIBSEM with Correlation to Cryo Fluorescence Microscopy** Harald Hess, Ph.D., Howard Hughes Medical Institute, Janelia Research Campus
1:50-2:20pm	**Subcellular Connectomic Analyses of the Energy Distribution System in Striated Muscle** Brian Glancy, Ph.D., National Heart, Lung, and Blood Institute and National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH
2:20-2:50pm	**Pre-Recorded Poster Presentations Segment #2**
2:50-3:20pm*	Afternoon Break
*2:50-3:20pm	**Poster Presenters Breakout Rooms**
3:20-3:50pm	**Tools for Analyzing and Controlling Biological Systems** Edward Boyden, Ph.D., Massachusetts Institute of Technology and Howard Hughes Medical Institute
3:55-4:25pm	**Metabolic Design of the Metabolic Extreme, the Shrew** Robert Balaban, Ph.D., National Heart, Lung, and Blood Institute, NIH
4:30-5:00pm	**Spatial Genomics: in situ Transcriptome Profiling by RNA seqFISH+** Long Cai, Ph.D., California Institute of Technology
5:05-5:35pm	**Integrating Single-DNA-Fiber Analyses and Genome-Wide Sequencing: Insights into the Dynamics of Chromosome Duplication** Mirit Aladjem, Ph.D., National Cancer Institute, NIH
5:35pm- 5:40pm	**Closing Remarks** Mark Knepper, M.D., Ph.D., National Heart, Lung, and Blood Institute, NIH
5:40pm	**Adjourn Day 1**

Agenda

Thursday, May 13, 2021

9:00-9:05am	**Opening Remarks** Keji Zhao, Ph.D. National Heart, Lung and Blood Institute, NIH

Session 3
Systems Physiology: Metabolomics and Sleep

Moderator: Hong Xu, Ph.D.

*Speaker Presentations slots are 25 minutes + 5 minutes Q&A + 5 minutes transition time

9:10-9:40am	**Identifying Metabolites That Alter Physiology** Gary Siuzdak, Ph.D., The Scripps Research Institute
9:45-10:15am	**Multi-Omic Approach to Understanding Regulation of Aquaporin-2 in Kidney** Mark Knepper, M.D., Ph.D., National Heart, Lung, and Blood Institute, NIH
10:15-10:45am	Morning Break
10:45-11:15am	**Obstructive Sleep Apnea and Cardiometabolic Disease: Dissecting Causal, Pleiotropic and Mediating Pathways** Susan Redline, M.D., M.P.H., Harvard Medical School, Brigham and Women's Hospital
11:20-11:50am	**Perturbing Genetic Networks That Influence Sleep Duration in *Drosophila Melanogaster*** Susan Harbison, Ph.D., National Heart, Lung, and Blood Institute, NIH
11:50am-12:20pm	**Pre-Recorded Poster Presentations Segment #3**
12:20-1:0. pm*	Lunch Break
*12:20-12:3. pm	**Poster Presenters Break-Out Rooms**

Agenda

Thursday, May 13, 2021

Systems Biology in Genetics and Development

Moderator: Brian Glancy, Ph.D.

*Speaker Presentations slots are 25 minutes + 5 minutes Q&A + 5 minutes transition time

1:20-1:50pm	**CRISPR Mediated Homologous Recombination and the Discovery of Rare Human Diseases** Hugo Bellen, DVM, Ph.D., Baylor College of Medicine and Neurological Research Institute
1:55-2:25pm	**scRNAseq Developmental Trajectories to Investigate Differentiation** Jeffrey Farrell, Ph.D., National Institute of Child Health and Human Development, NIH
2:25-2:40pm	Afternoon Break
2:40-3:10pm	**Single-Cell Transcriptomics of the Mouse Kidney Reveals Potential Cellular Targets of Kidney Disease** Katalin Susztak, M.D., Ph.D., University of Pennsylvania Perelman School of Medicine
2:15-3:45pm	**A Darwin Selection Within Developing Germ Cells** Hong Xu, Ph.D., National Heart, Lung, and Blood Institute, NIH
3:50-4:20pm	**A Systems-Wide Approach to Understanding Interorgan Communication in *Drosophila*** Norbert Perrimon, Ph.D., Harvard Medical School/Howard Hughes Medical Institute
4:25-4:55pm	**Mitochondrial Calcium and Regulation of Myocardial Cell Function** Elizabeth Murphy, Ph.D., National Heart, Lung, and Blood Institute, NIH
4:55 - 5:00pm	**Closing Remarks** Robert Balaban, Ph.D., Scientific Director, Division of Intramural Research National Heart, Lung, and Blood Institute, NIH
5:00pm	**Adjourn Day 2**

Scientific Organizing Committee

Scientific Organizing Committee

Keji Zhao, Ph.D. (Chair)
Director, Systems Biology Center
Chief, Laboratory of Epigenome Biology
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD
zhaok@nhlbi.nih.gov

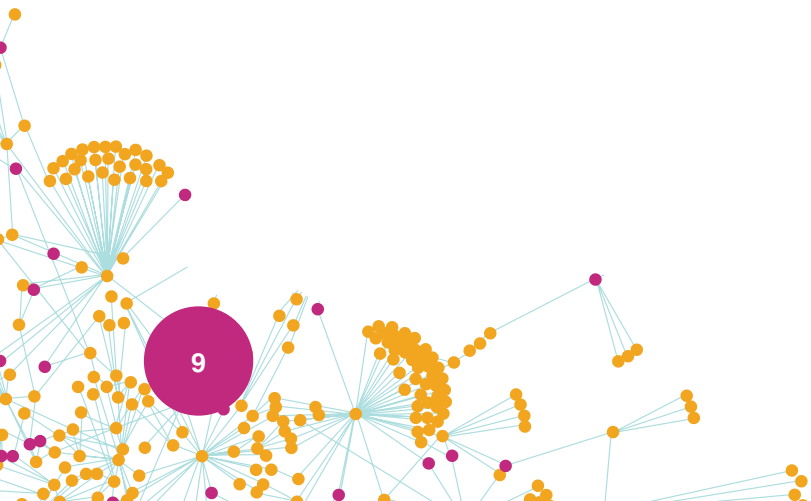
Robert S. Balaban, Ph.D.
Scientific Director, Division of Intramural Research
Chief, Laboratory of Cardiac Energetics
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD
balabanr@nhlbi.nih.gov

Brian Glancy, Ph.D.
Earl Stadtman Investigator
Chief, Muscle Energetics Laboratory
National Heart, Lung, and Blood Institute
National Institute of Arthritis and Musculoskeletal and Skin Disorders
National Institutes of Health
Bethesda, MD
glancybp@nhlbi.nih.gov

Susan T. Harbison, Ph.D.
Earl Stadtman Investigator
Chief, Laboratory of Systems Genetics
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD
susan.harbison@nih.gov

Mark A. Knepper, M.D., Ph.D.
Senior Investigator
Chief, Epithelial Systems Biology Laboratory
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD
knepperm@nhlbi.nih.gov

Hong Xu, Ph.D.
Senior Investigator
Chief, Laboratory of Molecular Genetics
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD
xuh5@mail.nih.gov



Invited Speakers

Invited Speakers

Mirit Aladjem, Ph.D.

Senior Investigator

Head, DNA replication Group,

Developmental Therapeutics Branch

Center for Cancer Research, NCI, NIH

Bethesda, MD

Dr. Aladjem received her Ph.D. from Tel Aviv University. She was a postdoctoral fellow and a Leukemia Society Special Fellow at the Salk Institute in La Jolla, California. Dr. Aladjem joined the NCI's Laboratory of Molecular Pharmacology / Developmental Therapeutics Branch in October 1999 and was appointed a Senior Investigator in 2007. Dr. Aladjem's studies focus on cellular signaling pathways that modulate chromatin to regulate chromosome duplication and cell cycle progression. Her team was the first to map replication origins on a whole genome scale, demonstrating a strong association between replication, histone modifications and chromatin packaging. Dr. Aladjem's current studies identify proteins that dictate whether particular chromatin regions would replicate during normal growth and after exposure to anti-cancer therapy. To further interactions with the scientific community, Dr. Aladjem co-chairs the NIH's Cell Cycle Interest Group and the NCI's Center of Excellence in Chromosome Biology.

Invited Speakers

Robert S. Balaban, Ph.D

Scientific Director, Division of Intramural Research

Chief, Laboratory of Cardiac Energetics

National Heart, Lung, and Blood Institute

National Institutes of Health

Bethesda, MD

balabanr@nhlbi.nih.gov

Robert S. Balaban, PhD, is the Scientific Director of the Division of Intramural Research (DIR) at the National Heart, Lung, and Blood Institute (NHLBI), part of the National Institutes of Health (NIH). In this role, Dr. Balaban oversees the NHLBI's laboratory and clinical research in heart, vascular, lung, blood, and kidney diseases. He also serves as the Chief of the NHLBI Laboratory of Cardiac Energetics. Prior to being named Scientific Director in 2004, Dr. Balaban served as a staff fellow in the NHLBI Laboratory of Kidney and Electrolyte Metabolism in 1982. In 1988 he was named Chief of the newly formed Laboratory of Cardiac Energetics. He was then appointed as Scientific Director of the Laboratory Research Program in 1999. Dr. Balaban received his Bachelor of Science in biology and chemistry from the University of Miami in 1971 and his Doctor of Philosophy in Physiology and Pharmacology from Duke University in 1980. He was also awarded a NATO fellowship to the Department of Biochemistry at the University of Oxford in 1981, where he studied the newly developed NMR methods to better understand intact biological systems.

Invited Speakers

Edward T. Boyden, Ph.D.

Y Eva Tan Professor in Neurotechnology

Massachusetts Institute of Technology

Howard Hughes Medical Institute

Cambridge, MA

edboyden@mit.edu

Ed Boyden is Y. Eva Tan Professor in Neurotechnology at MIT, an investigator of the Howard Hughes Medical Institute and the MIT McGovern Institute, and professor of Brain and Cognitive Sciences, Media Arts and Sciences, and Biological Engineering at MIT. He leads the Synthetic Neurobiology Group, which develops tools for analyzing and repairing complex biological systems such as the brain, and applies them systematically to reveal ground truth principles of biological function as well as to repair these systems. He co-directs the MIT Center for Neurobiological Engineering, which aims to develop new tools to accelerate neuroscience progress, and is a faculty member of the MIT Center for Environmental Health Sciences, Computational & Systems Biology Initiative, and Koch Institute. Amongst other recognitions, he has received the Wilhelm Exner Medal (2020), the Croonian Medal (2019), the Lennart Nilsson Award (2019), the Warren Alpert Foundation Prize (2019), the Rumford Prize (2019), the Canada Gairdner International Award (2018), the Breakthrough Prize in Life Sciences (2016), the BBVA Foundation Frontiers of Knowledge Award (2015), the Carnegie Prize in Mind and Brain Sciences (2015), the Jacob Heskel Gabbay Award (2013), the Grete Lundbeck Brain Prize (2013), the NIH Director's Pioneer Award (2013), the NIH Director's Transformative Research Award (three times, 2012, 2013, and 2017), and the Perl/UNC Neuroscience Prize (2011). He was also named to the World Economic Forum Young Scientist list (2013) and the Technology Review World's "Top 35 Innovators under Age 35" list (2006), and is an elected member of the National Academy of Sciences (2019), the American Academy of Arts and Sciences (2017), the National Academy of Inventors (2017), and the American Institute for Medical and Biological Engineering (2018). His group has hosted hundreds of visitors to learn how to use new biotechnologies, and he also regularly teaches at summer courses and workshops in neuroscience, and delivers lectures to the broader public (e.g., TED (2011), TED Summit (2016), World Economic Forum (2012, 2013, 2016)). Ed received his Ph.D. in neurosciences from Stanford University as a Hertz Fellow, working in the labs of Jennifer Raymond and Richard Tsien, where he discovered that the molecular mechanisms used to store a memory are determined by the content to be learned. In parallel to his PhD, as an independent side project, he co-invented optogenetic control of neurons, which is now used throughout neuroscience. Previously, he studied chemistry at the Texas Academy of Math and Science at the University of North Texas, starting college at age 14, where he worked in Paul Braterman's group on origins of life chemistry. He went on to earn three degrees in electrical engineering and computer science, and physics, from MIT, graduating at age 19, while working on quantum computing in Neil Gershenfeld's group. Long-term, he hopes that understanding how the brain generates the mind will help provide a deeper understanding of the human condition, and perhaps help humanity achieve a more enlightened state.

Invited Speakers

Hugo Bellen, D.V.M, Ph.D.

Professor of Genetics

Professor, Molecular and Human Genetics

Baylor College of Medicine and Neurological Research Institute

Houston, TX

hbellen@bcm.edu

My lab develops new tools to manipulate flies. We also typically generate the reagents for the fly community associated with each newly developed technology so that the methods can be used for most fly genes. The reagents that we have produced include more than 23,000 single transposable element insertion stocks in more than 80% of all fly genes distributed by the Bloomington Drosophila Stock Center (BDSC). We developed the P[acman] transgenic system that allows transformation of flies with genomic constructs up to 240 kB. We created and sequenced two widely used P[acman] genomic libraries with more than 100,000 clones that are distributed by BACPAC resources. We developed a novel transposable element named MiMIC that allows a staggering array of manipulations of fly genes and created 17,000 lines of which 7,500 have been deposited in the BDSC. We used these lines to tag 1,000 genes with a multifunctional GFP tag. In collaboration with Norbert Perrimon at Harvard, we have developed the CRIMIC technology. This allows us to insert a small MIMIC like cassette in almost any gene using CRISPR. The most commonly used tags SA-T2A-GAL4-poly A and SA-GFP-SD that are inserted in introns. The GAL4 tag typically creates a severe loss of function mutation and leads to the expression of GAL4 in the same temporal and spatial expression pattern as the gene that is tagged. This allows us to determine the expression pattern of the gene and drive fly or human cDNAs to rescue the induced mutation. We can then test fly or human variants for rescue and perform structure function studies. The SA-GFP-SD typically does not disrupt protein function and permits us to determine protein localization in vivo, Western blotting, immuno-precipitations, ChIP, TEM, and reversible protein inactivation in any tissue. My lab runs the fly Model Organisms Screening Center (MOSC) of the Undiagnosed Diseases Network (together with Shinya Yamamoto and Michael Wangler at BCM) to identify new genes and variants in human genes that cause disease using the technologies described above to humanize flies. We bring a unique expertise bridging the clinical practice of medical genetics with the mechanistic understanding that comes from studies of genetic models in Drosophila. We have helped in the identification of 30 novel human diseases in the past few years. I will present examples of recently identified genes and their mechanism of action as well as drugs that we identified.

Invited Speakers

Long Cai, Ph.D.

Professor of Biology and Biological Engineering
California Institute of Technology
Pasadena, CA
lcai@caltech.edu

Long Cai is a Professor of Biology and Biological Engineering at Caltech. His lab pioneered the field of spatial genomics by developing a method that allows the simultaneous imaging of over 10,000 genes in single cells within their native spatial context. This technology has opened a new way to directly visualize the genome in situ with microscopy, with many applications in neuroscience, stem cell biology, developmental biology and precision medicine. For this work, Dr. Cai has received the NIH New Innovator Award, Transformative Award, Paul G. Allen Frontiers Foundation Distinguished Investigator Award. Dr. Cai received his AB/AM in Physics and Chemistry at Harvard College, under the supervision of Dudley Herschbach, and his PhD in Chemistry at Harvard with Sunney Xie. He trained as a Beckman Institute Postdoctoral Fellow with Michael Elowitz at Caltech.

Invited Speakers

Jeffrey Farrell, Ph.D.

Earl Stadtman Investigator

Chief, Unit on Cell Specification and Differentiation

National Institute of Child Health and Human Development

National Institutes of Health

Bethesda, MD

jeffrey.farrell@nih.gov

Jeffrey Farrell is an Investigator in the National Institute for Child Health and Human Development's Intramural Research Program and started his independent research program in January 2020. His lab's research program focuses on understanding the genetic control of cell specification and differentiation during vertebrate embryogenesis. To do this, the Farrell lab uses single-cell RNAseq to investigate the cascades of gene expression that occur during development and then use reverse genetic screens, genomics, and imaging to elucidate the function of those genes.

The lab is interested in identifying the transcriptional programs that direct changes in cell biology during differentiation, their regulatory organization, and their reuse across cell types in development. The Farrell lab is also particularly interested in unappreciated inputs into cell specification programs, such as understanding how cell fate decisions are affected by developmental history (such as cells that change specification late in development) or environmental history (such as cells that experience DNA damage in the early embryo). Dr. Farrell has a broad background in the fields of developmental biology, genetics, and genomics. As a graduate student in Dr. Patrick O'Farrell's lab, he studied the developmental control of the cell cycle in early Drosophila embryos; there, he uncovered developmental regulation through Cdk1 downregulation that changes the replication timing of heterochromatic sequences in Drosophila embryos. As a post-doctoral fellow in Dr. Alexander Schier's lab, Dr. Farrell gained expertise in using zebrafish as a model organism and through a long-term collaboration with Dr. Aviv Regev's lab gained expertise in computational biology and single-cell RNA sequencing. There, he helped develop approaches to apply single-cell RNAseq to study developmental biology, including co-developing one of the first approaches for spatially assigning single-cell transcriptomes which was implemented in the first version of Seurat and developing the program URD for identifying branching transcriptional trajectories in single-cell RNAseq data.

Invited Speakers

Anna Greka, M.D., Ph.D.

Professor of Medicine

Harvard Medical School

Brigham and Women's Hospital

Broad Institute of MIT and Harvard

Boston, MA

agreka@broadinstitute.org

Anna Greka is an institute member of the Broad Institute of MIT and Harvard, where she leads a program focused on dissecting basic molecular and cellular mechanisms that may ultimately serve as the foundation for the development of targeted therapies. She is an associate professor at Harvard Medical School (HMS), and an associate physician in the Renal Division in the Department of Medicine at Brigham and Women's Hospital. The mission of the Greka laboratory is to define fundamental aspects of membrane protein biology and dissect mechanisms of cellular homeostasis. The laboratory complements this cell biology-focused program with tools from molecular biology, genomics, proteomics, and chemical biology. Combining expertise in ion channel biology with the study of kidney podocytes, the Greka laboratory uncovered a pathway linking TRPC5 ion channel activity to cytoskeletal dysregulation and cell death. Based on these discoveries, TRPC5 inhibitors are now being tested in the clinic for difficult-to-treat kidney diseases. More recently, the Greka laboratory made a key discovery of a general mechanism that monitors the quality of membrane protein cargoes destined for the cell surface by studying a proteinopathy in the kidney, caused by a mutation in MUC1. Specifically, the Greka lab identified a mechanism for membrane protein quality control that is operative in diverse cell types and tissues, such as kidney epithelial cells and retina photoreceptors. The study of cargo quality control is now a major focus of the laboratory. The Greka laboratory is also interested in dissecting the fundamental mechanisms of cellular homeostasis across the lifespan, with implications for many degenerative human diseases. Greka has been the recipient of several honors, including the 2020 Donald W. Seldin Young Investigator Award by the American Society of Nephrology and the American Heart Association, the 2018 Seldin-Smith Award for Pioneering Research from the American Society of Clinical Investigation, a 2017 Presidential Early Career Award for Scientists and Engineers, a 2014 Top 10 Exceptional Research Award from the Clinical Research Council, and a 2014 Young Physician-Scientist Award from the American Society of Clinical Investigation Council. She also serves on the Harvard-MIT M.D.-Ph.D. Program Leadership Council. Greka holds an A.B. in biology from Harvard College and an M.D. and Ph.D. in neurobiology from HMS. She received her medical and scientific training in the Harvard-MIT program in Health Sciences and Technology in the laboratory of National Academy of Sciences member David Clapham, where she explored the role of TRP channels in neuronal growth cone motility.

Invited Speakers

Susan T. Harbison, Ph.D.

Earl Stadtman Investigator

Chief, Laboratory of Systems Genetics

National Heart, Lung, and Blood Institute

National Institutes of Health

Bethesda, MD

susan.harbison@nih.gov

Susan T. Harbison, Ph.D., is an Earl Stadtman Tenure-Track Investigator at the National Heart, Lung, and Blood Institute. She investigates the genetic basis of sleep and circadian behavior in Drosophila melanogaster. Her research applies systems genetics and CRISPR transgenic approaches to reveal the genetic networks underlying sleep and to understand the forces that maintain genetic variation in these complex traits. Her goal is to determine how the genes in these networks interact with one another; how they respond to environmental perturbations; and to what degree they have been conserved across species.

Invited Speakers

Harald Hess, Ph.D.

Senior Group Leader

Howard Hughes Medical Institute

Janelia Research Campus

hessh@janelia.hhmi.org

After a PhD in Physics at Princeton in 1982, Harald Hess pursued cryogenic hydrogen atom trapping and its Bose-Einstein condensation at MIT as a postdoc and then developed various low temperature scanning probe microscopes to visualize diverse physics phenomenon at Bell Labs. After 1997 he spent 8 years in industry developing advanced equipment for hard disk drive and semiconductor inspection and production. In 2005, he and a colleague, Eric Betzig, learned about photoactivatable fluorescent proteins and invented PALM (photo-activated localization microscopy) to reveal details of cell structure beyond the diffraction limit. It was built in his La Jolla condo, tested at the National Institute of Health and perfected at Janelia Farms/Howard Hughes Medical Institute where he is extending PALM to a 3D super-resolution microscopy and exploring its application for cell biology research. He is also developing 3D electron microscopy techniques for volume imaging of cells and neural tissue as well as exploring modalities to correlate such electron microscopy with super-resolution microscopy.

Invited Speakers

Mark Knepper, M.D., Ph.D.

Senior Investigator

Chief, Epithelial Systems Biology Laboratory

National Heart, Lung, and Blood Institute

National Institutes of Health

Bethesda, MD

knepperm@nhlbi.nih.gov

Mark Knepper, M.D., Ph.D. is a Senior Investigator in the Systems Biology Center of the NHLBI. He is a biomedical engineer who uses systems biology-based approaches to study how the kidney regulates water excretion. This involves use of large-scale data acquisition techniques, such as protein mass spectrometry and 'next-generation' nucleotide sequencing technologies, coupled to computational techniques including mathematical modeling. Much of the focus of his laboratory, the Epithelial Systems Biology Laboratory, is on regulation of molecular water channels called "aquaporins" by the hormone vasopressin.

Invited Speakers

Elizabeth Murphy, Ph.D.

Senior Investigator

Chief, Laboratory of Cardiac Physiology

National Heart, Lung, and Blood Institute

National Institutes of Health

Bethesda, MD

murphy1@mail.nih.gov

Dr. Elizabeth Murphy is a Chief of the Cardiac Physiology Section in the Cardiovascular Branch at NHLBI, Bethesda, MD USA. She received her PhD from the University of Pennsylvania in Biochemistry, followed by postdoctoral studies in Physiology at Duke University. Her research is focused on ionic, redox and energetic alterations in cell death and cardioprotection. She has also focused on mechanisms regulating sex differences in cardiovascular disease. She is a Fellow of the ISHR and the American Heart Association (AHA) and serves as Deputy Editor of Circulation Research. She is also the North American Coordinator of a Leducq Transatlantic Network of Excellence on Targeting Mitochondria to Treat Heart Disease.

Invited Speakers

Norbert Perrimon, Ph.D.

Professor of Genetics

Blavatnik Institute, Harvard Medical School

Howard Hughes Medical Institute

Boston, MA

perrimon@genetics.med.harvard.edu

Norbert Perrimon is the James Stillman Professor of Developmental Biology in the Department of Genetics at Harvard Medical School, an Investigator of the Howard Hughes Medical Institute, and an Associate member of the Broad Institute. His laboratory has pioneered a number of techniques in Drosophila such as the FLP-FRT Dominant Female Sterile technique to generate germline mosaics, the GAL4-UAS method to control gene expression both spatially and temporally, highthroughput genome wide RNAi and CRISPR screens, and proximity labeling methods to identify secreted molecules.

These methods have had transformative impacts in signal transduction, development, physiology, neurobiology, and functional genomics. Early in his career, he identified and characterized factors involved in RTK, Wnt, and JAK/STAT signaling, contributing to the elucidation of these canonical pathways. Perrimon went on discover intestinal stem cells in the adult fly gut, opening up an entire field of study to identify factors and pathways involved in stem cell homeostasis and regeneration. In more recent years, he has taken a systematic approach to identify factors involved in inter-organ communication, which are leading to a systems wide understanding of how hormonal systems are regulated by the state of various organs in homeostatic and stressed conditions. Perrimon has been on the faculty of Harvard Medical School since 1986. He received the George W. Beadle Medal form the Genetics Society of America in. 2004. He has been elected to the American Academy of Arts and Sciences, American Association for the Advancement of Science, EMBO, and National Academy of Sciences. He has trained more than 110 students and postdoctoral fellows, with most of them currently holding academic positions.

Invited Speakers

Susan Redline, M.D., M.P.H.

Peter C. Farrell Professor of Sleep Medicine

Brigham and Women's Hospital

Harvard Medical School

Boston, MA

sredline@bwh.harvard.edu

Susan Redline, MD, MPH, is the Peter C. Farrell Professor of Sleep Medicine at Harvard Medical School and Director of the Programs in Sleep and Cardiovascular Medicine and Sleep Medicine Epidemiology at Brigham and Women's Hospital, Harvard Medical School. She directs the Sleep Reading Centers for a number of major cohort studies and clinical trials and co-directs the National Sleep Research Resource, a large repository of research data and tools to promote sleep research. She directs the Data Coordinating Centers for several NIH-funded multi-center clinical trials.

Her research addresses 1) the etiologies of sleep disorders, including the role of genetic and early life developmental factors and health disparities; and 2) the cardiovascular and other health outcomes of sleep disorders and the role of sleep interventions in improving health.

Invited Speakers

Gary Siuzdak, Ph.D.

Professor of Chemistry, Molecular and Computational Biology

Director, Scripps Center for Metabolomics

The Scripps Research Institute

La Jolla, CA

siuzdak@scripps.edu

Gary Siuzdak is Professor and Director of the Scripps Center for Metabolomics at The Scripps Research Institute. He is an affiliate scientist at the Lawrence Berkeley National Lab and has served as Vice President of the American Society for Mass Spectrometry. His research includes developing novel technologies for metabolomics, metabolite imaging and systems biology. He has hundreds of peer-reviewed publications and two books including the “The Expanding Role of Mass Spectrometry in Biotechnology”.

Invited Speakers

Katalin Susztak, M.D., Ph.D.

Professor of Medicine and Genetics
University of Pennsylvania
Perelman School of Medicine
Philadelphia, PA
ksusztak@pennmedicine.upenn.edu

Dr. Katalin Susztak is a physician scientist. She is a professor of Medicine and Genetics at the University of Pennsylvania. Susztak has made discoveries fundamental towards defining critical genes, cell types and mechanisms of chronic kidney disease. She was instrumental in defining genetic, epigenetic transcriptional changes in diseased human kidneys. She identified multiple novel kidney disease genes and demonstrated role of Notch signaling and metabolic dysregulation in kidney disease development. Her lab was the first to map the kidney epigenome and catalogue genotype-driven gene-expression variation (eQTL) in human kidneys. Integration of GWAS, eQTL and epigenome data has been essential to prioritize disease-causing genes and variants. Dr. Susztak generated the first unbiased, comprehensive kidney cell-type atlas using single cell transcriptomics. She identified that specific renal endophenotypes are linked and likely caused by the dysfunction of specific cell types. In follow-up animal model studies, she conclusively demonstrated that MANBA, DAB2, DACH1 and APOL1 are new kidney disease risk genes. Her work established the role of proximal tubule cells, endolysosomal trafficking, metabolic and developmental pathways in kidney disease development. Susztak's discoveries span genetics, genomics, epigenetics, molecular biology, physiology and nephrology, and have enormous translational relevance and considerable therapeutic potential.

Invited Speakers

Xiaoliang Sunney Xie, Ph.D.

Lee Shau-kee Professor
Peking University
Beijing, China
sunneyxie@pku.edu.cn

Professor Xiaoliang Sunney Xie is the Lee Shau-kee Professor of Peking University. He is member of the U.S. National Academy of Sciences, U.S. National Academy of Medicine, American Academy of Arts and Sciences, and Foreign Member of the Chinese Academy of Sciences. He was the Mallinckrodt Professor of Chemistry and Chemical Biology
at Harvard University until 2018. He is currently Director of Biomedical Pioneering Innovation Center at Peking University and Director of Beijing Advanced Innovation Center for Genomics. As a pioneer of single-molecule biophysical chemistry, coherent Raman scattering microscopy, and single-cell genomics, he made major contributions to the emergence of these fields. In particular, his inventions in single-cell genomics have been used in in vitro fertilization to benefit thousands of couples in China by avoiding the transmission of monogenic diseases to their newborns. Prof. Xie received numerous international awards, in particular, Albany Prize in Medicine and Biomedical Research, Peter Debye Award of American Chemical Society and Biophysical Society Founders Award.

Invited Speakers

Hong Xu, Ph.D.

Senior Investigator

Chief, Laboratory of Molecular Genetics

National Heart, Lung, and Blood Institute

National Institutes of Health

Bethesda, MD

xuh5@mail.nih.gov

Hong Xu, Ph.D., is a Senior Investigator at the National Heart, Lung and Blood Institute, studying the second genome of eukaryotes-mitochondrial DNA. Over the years, the lab have been developed various tools to manipulate mitochondrial genome. They have been applying these tools together with the powerful genetic resources for nuclear genome to understand the basic principles and cellular processes guiding mtDNA transmission. Dr. Xu received Ph.D. from John University in 2004 and did postdoctoral training at University of California San Francisco, before joining NHLBI as a tenure-track Investigator in 2010.

Invited Speakers

Richard Young, Ph.D.

Professor of Biology

Whitehead Institute for Biomedical Research

Massachusetts Institute of Technology

Cambridge, MA

young@wi.mit.edu

Richard Young studies gene regulation in health and disease. He has served as an advisor to the World Health Organization, the National Institutes of Health and numerous scientific societies and journals. Dr. Young's honors include Membership in the National Academy of Sciences and the National Academy of Medicine, and Scientific American has recognized him as one of the top 50 leaders in science, technology and business. He has founded and advised companies in the biotechnology and pharmaceutical industry, and currently serves on the boards of Syros Pharmaceuticals, CAMP4 Therapeutics, Omega Therapeutics and Dewpoint Therapeutics.

Invited Speakers

Keji Zhao, Ph.D.

Director, Systems Biology Center

Chief, Laboratory of Epigenome Biology

National Heart, Lung, and Blood Institute

National Institutes of Health

Bethesda, MD

zhaok@nhlbi.nih.gov

Keji Zhao, Ph.D., is director of the Systems Biology Center at the NHLBI, as well as a senior investigator in the Laboratory of Epigenome Biology. Dr. Zhao joined the NHLBI in 1999 and has been a senior investigator since 2007. He was elected to the rank of AAAS Fellow in 2012. Dr. Zhao received his undergraduate degree from Changwei Normal College in Weifang, China in 1980, Master of Science degree from Northeast Normal University, Changchun, China in 1985, and his Doctor of Philosophy from the University of Geneva, Switzerland in 1996. Prior to joining the NHLBI, Dr. Zhao was a Damon Runyon-Walter Winchel Cancer Research Postdoctoral Fellow at Stanford University, California.

Dr. Zhao's research focuses on the epigenetic regulation of chromatin. Understanding how epigenetic patterns are established during development and how improper epigenetic signals contribute to disease is the long-term goal for his lab. His laboratory developed many -Seq techniques, including ChIP-SAGE, ChIP-Seq, MNase-Seq, single-cell DNase-seq (scDNase-seq), single-cell MNase-seq, single-cell ChIC-seq, ACT-seq, and TrAC-looping, and also developed corresponding algorithms to analyze these data. Using these approaches, Dr. Zhao's lab pioneered whole-genome analyses of chromatin modifications in higher eukaryotic systems. Dr. Zhao's laboratory is currently investigating the function and regulation of three-dimensional nuclear structure and epigenomic cellular heterogeneity in the immune system.

Speaker Abstracts

Speaker Abstracts

Table of Contents

Table of Contents (cont.)

Speaker Abstracts

Metabolic design of the metabolic extreme, the Shrew

Dillon J. Chung[1]*, Grey P. Madison[1], Angel M. Aponte[1], Komudi Singh[1], Yuesheng Li[1], Mehdi Pirooznia[1], Christopher K. E. Bleck[1], Nissar A. Darmani[2] and Robert S. Balaban[1]

[1]National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]Department of Basic Medical Sciences, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, Pomona, CA

*Corresponding author. Email: dillon.chung@nih.gov

Mitochondrial adaptations are fundamental to support differentiated function and energetic homeostasis in mammalian cells. Organ-specific mitochondrial adaptations were investigated in the metabolic extreme, Cryptotis parva, using 3D electron microscopy and protein composition. C. parva liver and kidney mitochondrial content were equivalent to the heart permitting assessment of mitochondrial adaptations in different organs with similar metabolic demand. Muscle mitochondrial demonstrated extensive network reticulum with specialized structures apparently coupling large single mitochondria domains. In sharp contrast, mitochondrial networks were detected in the liver and kidney despite having similar mitochondria concentrations. In liver and kidney, individual mitochondria are localized with sites of ATP consumption (i.e. kidney: basolateral membrane Liver: endoplasmic reticulum) which is not achievable in striated muscle due to a homogenous ATPase distribution. These results demonstrate distinct mitochondrial structural adaptations for similar overall metabolic need that match the distribution of energy utilization processes. The two basic models observed so far are: 1)Interconnected network of mitochondria to support diffuse ATPase activity and 2) Individual mitochondria placed adjacent to highly localized ATPase activity to locally provided a balanced energy conversion and utilization without a interconnecting network.

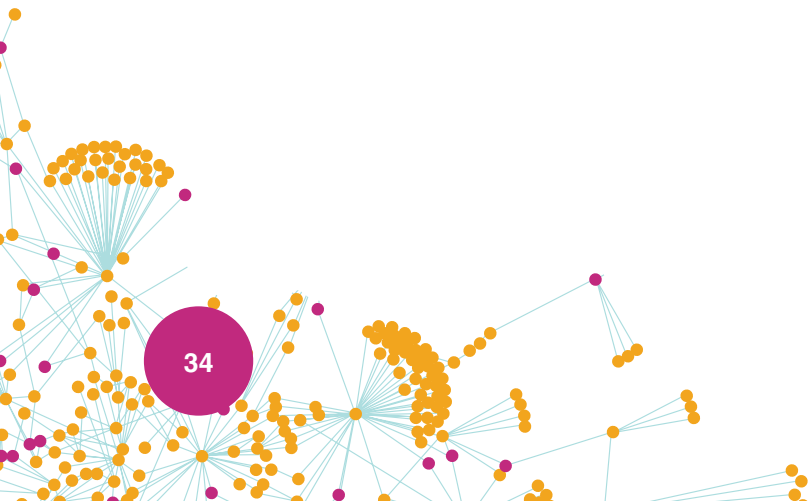
Speaker Abstracts

Tools for Analyzing and Controlling Biological Systems

Edward Boyden, Ph.D.

Massachusetts Institute of Technology and Howard Hughes Medical Institute, Cambridge, MA

Understanding and repairing complex biological systems requires technologies for systematically observing and controlling these systems. We are discovering new molecular principles that enable such technologies. For example, we discovered that one can physically magnify biological specimens by synthesizing dense networks of swellable polymer throughout them, and then chemically processing the specimens to isotropically swell them. This method, which we call expansion microscopy, enables ordinary microscopes to do nanoimaging. Expansion of biomolecules away from each other also decrowds them, enabling previously invisible nanostructures to be labeled and seen. As a second example, we are developing, using new strategies such as robotic directed evolution, fluorescent reporters that enable the precision measurement of signals. By fusing such reporters to self-assembling peptides, they can be stably clustered within cells at random points, distant enough to be resolved by a microscope, but close enough to spatially sample the relevant biology. Such clusters, which we call signaling reporter islands (SiRIs), permit many fluorescent reporters to be used within a single cell, to simultaneously reveal relationships between different signals.



Speaker Abstracts

CRISPR mediated homologous recombination and the discovery of rare human diseases

Oguz Kanca[1], Jonathan Zirin[2], Claire Yanhui Hu[2], Shinya Yamamoto[1], Michael F. Wangler[1], Susan E. Celniker[4], Stephanie Mohr[2], Robert W. Levis[3], Allan C. Spradling[3,5], Nobert Perrimon[2,5], Hugo J. Bellen[1,5]

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX

[2]Department of Genetics, Harvard Medical School, Boston, MA

[3]Department of Embryology, Carnegie Institution for Science, Baltimore, MD

[4]Berkeley Drosophila Genome Project, Lawrence Berkeley National Laboratory, Berkeley, CA

[5]Howard Hughes Medical Institute

Despite increasing knowledge about genomes and genetic variation, experimental knowledge about the function of numerous genes is still limited. Drosophila offers the strongest genetic tool set to study gene function. The Drosophila Gene Disruption Project (GDP) aims to provide genetic tools to study the function of genes that are conserved. We use CRISPR mediated homologous recombination to integrate cassettes in the introns of genes (CRIMIC). They typically contain a mutagenic region like a Splice Acceptor-T2A-GAL4-polyA and a dominant marker flanked by attP sites to allow Recombinase Mediated Cassette Exchange. This generates a severe loss of function allele, while expressing a GAL4 with the expression pattern of the targeted gene. To date, the GDP has integrated casettes in introns of more than 2,000 genes and has converted these casettes into diverse genetic tools. In addition, we are generating a comprehensive UAS-human cDNA transgenic fly library to facilitate gene function studies. These UAS-human cDNA transgenes can be driven by T2A-GAL4 to rescue their associated phenotypes, replacing the fly gene with the orthologous human gene. This has greatly helped in the discovery of many human rare diseases identified by human geneticist, including many genes identified in the Undiagnosed Diseases Network.

Speaker Abstracts

Spatial Genomics: in situ Transcriptome Profiling by RNA seqFISH+

Long Cai, Ph.D.

California Institute of Technology, Pasadena, CA

Imaging the transcriptome in situ with high accuracy has been a major challenge in single cell biology, particularly hindered by the limits of optical resolution and the density of transcripts in single cells. We developed seqFISH+, that can image the mRNAs for 10,000 genes in single cells with high accuracy and sub-diffraction-limit resolution, in the mouse brain cortex, subventricular zone, and the olfactory bulb, using a standard confocal microscope. The transcriptome level profiling of seqFISH+ allows unbiased identification of cell classes and their spatial organization in tissues. In addition, seqFISH+ reveals subcellular mRNA localization patterns in cells and ligand-receptor pairs across neighboring cells. This technology demonstrates the ability to generate spatial cell atlases and to perform discovery-driven studies of biological processes in situ.

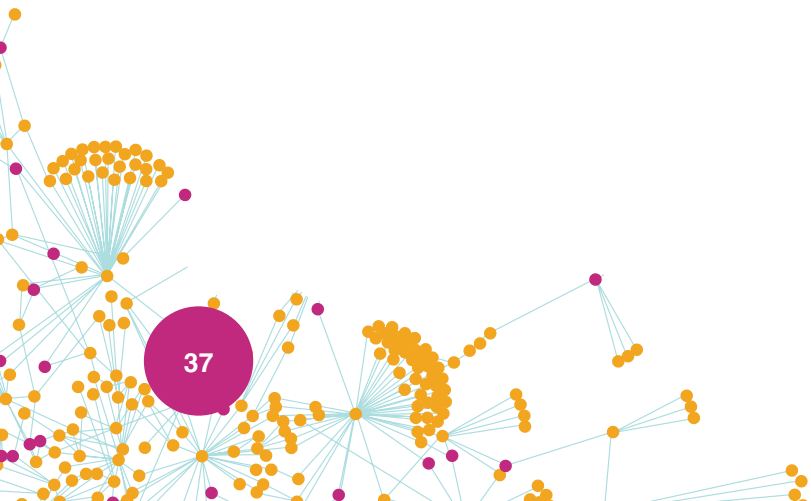
Speaker Abstracts

scRNAseq Developmental Trajectories to Investigate Differentiation

Abhinav Sur, Yiqun Wang, Gennady Margolin, Aviv Regev, Alex Schier, Jeffrey Farrell

National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD

A crucial quest in developmental biology is to understand the sequence of gene expression in each cell type as it is specified and differentiates and to understand how those genes shape cells' characteristics. To uncover such gene expression cascades from single-cell RNA sequencing data, we previously developed the semi-supervised computational approach URD. URD uses biased random walks through transcriptomic data to build a branching tree of the paths cells take in gene expression as they develop. I will discuss how we have applied this approach to identify the developmental trajectories and gene expression cascades during zebrafish embryogenesis. We are now focused on using these data to identify gene expression programs that prepare for and execute cellular remodeling during differentiation. Our initial efforts have focused on understanding the organization of the gene expression cascades within the notochord and prechordal plate, two cell types that share a common progenitor. Both cell types become highly secretory during differentiation and prepare themselves for this cellular stress in advance. We find that these cell types employ a core transcriptional program (the unfolded protein response), but each cell type uses specific regulators to customize this response for its particular secretory needs.



Speaker Abstracts

Subcellular Connectomic Analyses of the Energy Distribution System in Striated Muscle

Brian Glancy, Ph.D.

Muscle Energetics Laboratory, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), and National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), Bethesda, MD

Cellular development requires the integrated assembly of intracellular structures into functionally specialized regions supporting overall cellular performance. However, it remains unclear how coordination of organelle interactions contributes to the development of functional specificity across cell types. In striated muscles, continued muscle contraction is supported in large part by energy metabolism and calcium cycling. Thus, knowledge of the interactions between mitochondria, which are central to both of these processes, and the cellular sites of energy storage, utilization, and calcium uptake and release is critical for our understanding of muscle function. By combining focused ion beam scanning electron microscopy and machine learning image segmentation, we have developed a network scale analytical framework to quantitatively assess the three-dimensional physical interactions among striated muscle organelles (mitochondria, lipid droplets, sarcoplasmic reticulum (SR), etc.) and the muscle contractile apparatus (myofibrils, sarcomeres, myosin filaments, etc.). Utilizing this subcellular connectomics approach, we show that the physical interactions within mitochondria and contractile networks as well as among different cellular structures (e.g. mitochondria-SR contacts) are specifically tuned to support muscle cell function in a muscle type (e.g. slow-twitch vs. fast-twitch) and developmentally regulated manner.

Speaker Abstracts

Integrative Approaches Probing Disrupted Cellular Homeostasis in Metabolic Diseases

Anna Greka, M.D., Ph.D.

Harvard Medical School, Brigham and Women's Hospital, Broad Institute of MIT and Harvard, Boston, MA

The mission of the Greka laboratory is to define fundamental aspects of membrane protein biology and dissect mechanisms of cellular homeostasis. The laboratory complements this cell biology-focused program with tools from molecular biology, genomics, proteomics, and chemical biology.

Combining expertise in ion channel biology with the study of kidney podocytes, the Greka laboratory uncovered a pathway linking TRPC5 ion channel activity to cytoskeletal dysregulation and cell death. Based on these discoveries, TRPC5 inhibitors are now being tested in the clinic for difficult-to-treat kidney diseases.

More recently, the Greka laboratory made a key discovery of a general mechanism that monitors the quality of membrane protein cargoes destined for the cell surface by studying a proteinopathy in the kidney, caused by a mutation in MUC1. Specifically, the Greka lab identified a mechanism for membrane protein quality control that is operative in diverse cell types and tissues, such as kidney epithelial cells and retina photoreceptors. The study of cargo quality control is now a major focus of the laboratory.

The Greka laboratory is also interested in dissecting the fundamental mechanisms of cellular homeostasis across the lifespan, with implications for many degenerative human diseases.

Speaker Abstracts

Perturbing genetic networks that influence sleep duration in Drosophila melanogaster

Susan T. Harbison, Ph.D.

Laboratory of Systems Genetics, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI)
National Institutes of Health (NIH), Bethesda, MD

An increasing appreciation of the negative impact that sleep disruption and sleep disorders have on human health has motivated studies to understand the underlying genetic basis of sleep. However, sleep is a complex trait influenced by a large genetic network and remains refractory to standard techniques. This talk will describe how an artificial selection approach in flies can identify genomic sequence variants and co-regulated transcriptional networks affecting sleep duration. An evaluation of the conservation of function across species pinpoints the most critical genetic targets. Extreme-sleeping inbred lines developed from the artificially-selected populations become the substrate for single- and multiple-allelic replacement using CRISPR techniques. Combinatorial perturbations to the genetic network then reveal the functional role of each target gene.

Speaker Abstracts

3D Imaging of Cells by FIBSEM with Correlation to Cryo Fluorescence Microscopy

Harald Hess, Ph.D.

Howard Hughes Medical Institute, Janelia Research Campus, Ashburn, VA

3D electron microscopy data can be acquired by Focused Ion Beam Scanning Electron Microscopy FIB-SEM where fine sequence of 4-8 nm increments are ablated off of a sample surface and each such surface is imaged with the SEM. At the finest resolution and with month long stable operation, comprehensive whole cells can be acquired that transcends the limited cut section views of traditional TEM used in biology. Several examples of such data are presented along with the potential that segmentation offers to explore and formulate biological questions. Correlative microscopy can be achieved by a cryogenic protocol where samples are vitrified, imaged with PALM or SIM at low temperatures followed by EM staining and FIBSEM. A 3D registration procedure can keep most position errors between PALM and EM data at ~ 30 nm. Examples validating the approach with mitochondrial and endoplasmic reticulum labels are presented along with examples showcasing how unknown vesicle types and other structures can be identified by an associated protein.

Speaker Abstracts

Multi-omic Approach to Understanding Regulation of Aquaporin-2 in Kidney

Mark Knepper, M.D., Ph.D.

Epithelial Systems Biology Laboratory, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Aquaporin-2 (AQP2) is selectively expressed in the kidney collecting duct and is responsible for vasopressin-mediated control of water excretion. It is regulated by vasopressin through: (1) control of trafficking of AQP2-containing membrane vesicles to and from the plasma membrane; and (2) control of transcription of the Aqp2 gene. Both protein mass spectrometry (proteomics) and NGS modalities are being applied to understand the mechanisms that link the vasopressin V2 receptor to regulation of Aqp2 gene transcription. The V2 receptor is a Gs coupled receptor that signals largely through increases in cAMP. CRISPR deletion of both genes that code for PKA catalytic subunits (Prkaca and Prkacb) followed by RNA-seq and proteomics in a vasopressin-responsive cell line demonstrated a highly selective loss of AQP2 mRNA and protein. Vasopressin treatment of wild type cells produced a mirror-image response with increased AQP2 mRNA and protein. RNA polymerase II occupancy over the Aqp2 gene body was increased (ChIP-seq). ATAC-seq combined with histone H3K27Ac ChIP-seq identified five regions in the Aqp2-containing CTCF loop that identify potential enhancers. Among candidate transcription factors with potential binding sites in these regions, C/EBP-b was found by ChIP-seq to bind to a predicted site and binding was increased by vasopressin.

Speaker Abstracts

A Systems-wide Approach to Understanding Interorgan Communication in Drosophila

Norbert Perrimon, Ph.D.

Department of Genetics, Blavatnik Institute, Harvard Medical School/
Howard Hughes Medical Institute, Boston, MA

I will discuss our ongoing studies to identify and characterize communication pathways between major organs in Drosophila. Organ-to-organ communications are critical to living systems and play major roles in homeostasis. For example, the vertebrate CNS receives information regarding the status of peripheral metabolic processes via hormonal signaling and direct macromolecular sensing. In addition, skeletal muscles produce various myokines that influence metabolic homeostasis, lifespan, and the progression of age-related diseases and aging in non-muscle tissues. Using genetic screening, transcriptome analyses and proteomic approaches, we are identifying and characterizing secreted factors by which organs communicate their physiological state to others in both homeostasis and altered metabolism, as well as tumor models. These studies are providing fundamental insights into how biological processes observed in one tissue/organ (e.g., decreased cellular metabolism, mitochondrial dysfunction) influence the state of other tissues/organs.

Speaker Abstracts

Obstructive sleep apnea and cardiometabolic disease: Dissecting causal, pleiotropic and mediating pathways

Susan Redline, M.D., M.P.H.

Harvard Medical School, Brigham and Women's Hospital, Boston, MA

Obstructive sleep apnea (OSA) is a prevalent but complex and heterogeneous disorder characterized by nightly breathing pauses that result in intermittent hypoxemia, sleep fragmentation, and autonomic nervous system dysfunction. These stressors can activate circulating white blood cells and contribute to insulin resistance, dyslipidemia and endothelial dysfunction, as well as promote angiogenesis. Conversely, inflammation may increase risk for OSA via effects on ventilation and upper airway patency. Dissecting etiological mechanisms driving susceptibility to OSA vs those that mediate cardiometabolic disease requires multi-level analyses of genomic data and physiological endotypes; use of complementary observational and intervention designs; and which leverages tools such as mendelian randomization and polygenic risk scores (PRS). This talk highlights (1) the complex etiology and physiology of OSA, including prominent gender differences; (2) Initial signals (genetic association, transcriptome, methylation) obtained from genomic analyses pointing to OSA-associated dysregulation of inflammatory and heme-related pathways; and (3) A novel pathway-specific PRS for testing OSA as moderator for cardiovascular disease. System-level studies of OSA may elucidate key mechanisms for chronic disease, including pathways that are protective as well as adverse; help identify subgroups at differing risk for OSA-related cardiometabolic disease; and identify potential novel intervention targets.

Speaker Abstracts

Identifying Metabolites that Alter Physiology

Markus M. Rinschen[1], Julijana Ivanisevic[2], Martin Giera[3] and Gary Siuzdak[1]

[1]The Scripps Research Institute, Center for Metabolomics and Mass Spectrometry, La Jolla, CA, USA

[2]Metabolomics Platform, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland

[3]Leiden University Medical Center, Center for Proteomics & Metabolomics, Leiden, Netherlands

The metabolome, the collection of small-molecule chemical entities involved in metabolism, has traditionally been studied with the aim of identifying biomarkers in the diagnosis and prediction of disease. However, the value of metabolome analysis (metabolomics) has been redefined from a simple biomarker identification tool to a technology for the discovery of active drivers of biological processes. It is now clear that the metabolome affects cellular physiology through modulation of other 'omics' levels, including the genome, epigenome, transcriptome and proteome. In this presentation, I will focus on recent progress in using metabolomics to understand how the metabolome influences other omics and, by extension, to reveal the active role of metabolites in physiology and disease. This concept of utilizing metabolomics to perform activity screens to identify biologically active metabolites — which we term activity metabolomics — is already having a broad impact on biology.

Speaker Abstracts

Single-cell Transcriptomics of the Mouse Kidney Reveals Potential Cellular Targets of Kidney Disease

Katalin Susztak M.D., Ph.D.

University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA

The functional interpretation of GWAS remains challenging due to cell-type dependent influences of genetic variants.

We generated comprehensive maps of expression quantitative trait loci (eQTL) for 659 microdissected human kidney samples and identified cell-type eQTLs by mapping interactions between cell type abundance and genotype. Separately, we generated single cell open chromatin maps (by snATAC-seq) for human kidney samples. We highlight critical enrichment of proximal tubules in kidney function and endothelial cells and distal tubule segments in blood pressure by partitioning heritability using stratified LD-score regression to integrate GWAS with scRNA-seq and snATAC-seq data. Bayesian colocalization analysis nominated more than 200 genes for kidney function and hypertension. Our study clarifies the mechanism of the most commonly used antihypertensive and renal protective drugs and identifies drug repurposing opportunities for kidney disease.

Speaker Abstracts

New Advances in Single-cell 3D Genome Structures and High Precision Transcriptomics

Xiaoliang Sunney Xie, Ph.D.

Peking University

No abstract provided

Speaker Abstracts

A Darwin Selection Within Developing Germ Cells

Hong Xu, Ph.D.

Laboratory of Epigenome Biology, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Mitochondria power eukaryotes' lives, also leave an Achilles Heel-mitochondrial genome that encodes key components of oxidative phosphorylation system. Mitochondrial genome is prone to accumulating mutations, which could severely impair energy metabolism and potentially lead to the genetic meltdown of mitochondria and the extinction of eukaryotes. It has been long debated, but remains unsettled how organisms are able to avoid the accumulation of mitochondrial mutations. Mitochondria are transmitted exclusively through maternal lineage in metazoan. I will talk about a series of developmentally-orchestrated processes that limits the transmission of mitochondrial mutations in the female germline of fruit flies.

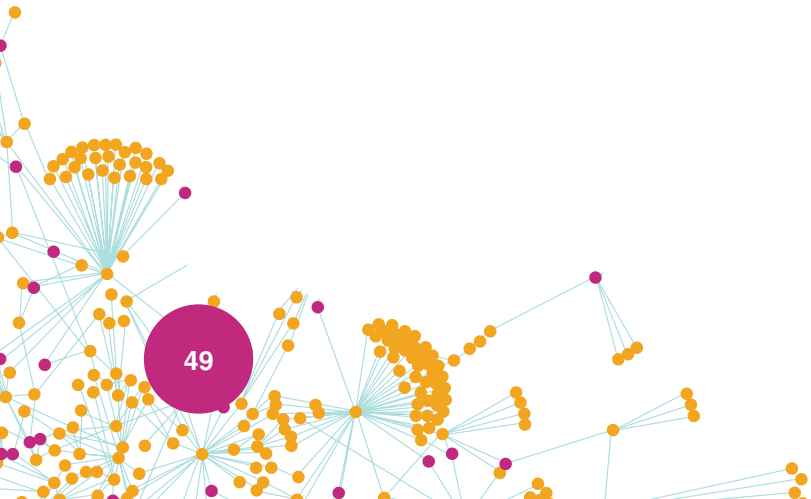
Speaker Abstracts

Nuclear Condensates in Gene Regulation and Disease

Richard Young, Ph.D.

Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology

Nuclear processes such as chromosome maintenance and transcription depend on the concerted action of many protein and RNA molecules. Recent studies have shown that many nuclear processes occur within biomolecular condensates, which compartmentalize the community of protein and RNA molecules involved in each process, typically at specific genomic loci. I will discuss the features of condensates that provide the cell with regulatory capabilities beyond canonical molecular regulatory mechanisms, note where these are dysregulated by pathological mutations, and explain how our new understanding of chemical partitioning is influencing the development of new therapeutics for cancer and other diseases.



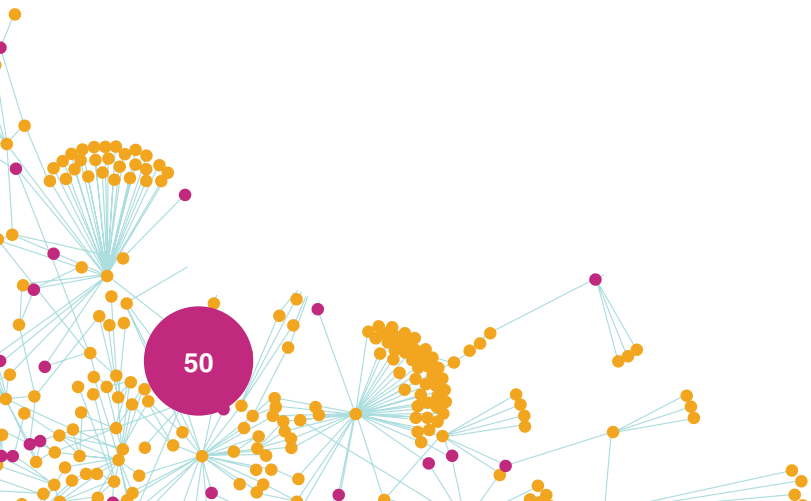
Speaker Abstracts

Epigenetic basis of single-cell heterogeneity

Keji Zhao, Ph.D.

Laboratory of Epigenome Biology, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Single-cell profiling of gene expression has revealed substantial heterogeneity in expression levels among phenotypically indistinguishable cells. This variability has important functional implications for tissue biology and disease states such as cancer. Mapping of epigenomic information such as chromatin accessibility, nucleosome positioning, histone tail modifications, and enhancer-promoter interactions in both bulk- and single-cell samples have shown that these characteristics of chromatin state contribute to expression or repression of associated genes. Our recent studies using single-cell epigenomics techniques provide evidence that variations in different aspects of chromatin organization collectively define gene expression heterogeneity among otherwise highly similar cells.



Pre-Recorded Poster Presentations

Segment 1

Pre-Recorded Presentations

Table of contents

Segment 1

Pre-Recorded Presentations

Table of contents

Segment 2

Pre-Recorded Presentations

Table of contents

Segment 3

Abstract 1

Characterising early T cell activation using Single Cell Pathway Analysis (SCPA)

Jack Bibby[1], Divyansh Agarwal[2], Natalia Kunz[1], Tilo Freiwald[3], Erin West[1], Behdad Afzali[3], Claudia Kemper[1], Nancy Zhang[2]

[1]Laboratory for Complement and Inflammation Research, Immunology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]Statistics Department, The Wharton School, University of Pennsylvania, Philadelphia, PA

[3]Kidney Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Bethesda, MD

Pathway analysis provides a powerful method for discerning the most biologically relevant signals in omics datasets. Current methods are based on the assumption that mean changes in gene expression are the most meaningful metric for assessing pathway importance, which significantly undervalues the complexity of scRNA-seq data. We therefore developed a fundamentally novel method of pathway analysis whereby biological interest is primarily understood as a change in multivariate distribution of a given pathway. To gain insights into gene set dynamics over T cell activation and differentiation, we generate a comprehensive scRNA-seq T cell resource from sorted and stimulated T cells, and apply SCPA across multiple scenarios. In doing so, we identify distinct biological signatures across time and between T cell populations, including differential regulation of type I interferon responses, and a systems level understanding of metabolic reprogramming. Within the analysis, we highlight multiple features of SCPA, including classical two-sample comparisons, multisample pathway analysis, and pathway analysis over a pseudotime trajectory. Finally, we perform a comprehensive analysis of pathway signatures in over 25 cell types from the blood and lung of COVID-19 patients. Our work here provides a key T cell resource, alongside a robust and sensitive method for assessing pathway activity

Abstract 2

Detection of immune response changes through multiomics temporal analysis

Minzhang Zheng and George Mias

Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

Background: Identifying common individual-level changes in physiological states across a multi-person cohort, by comparing individualized temporal monitoring of multiple omics, presents several challenges for clinical implementations. We address issues such as multiple measurements across different scales, missing datapoints, and uneven sampling using novel network-based analyses of multi-omics, including inter- and intra- individual changes, and explore immune responses in individualized multiomics from healthy and diabetic individuals.

Methods: We apply spectral methods to analyze multiomics individual profiles from public data for 69 individuals. We generate periodograms for individual subject omics signals, to construct within-person omics networks and analyze personal-level immune changes. We use periodograms across individuals to identify network clusters of individuals with similarities across their common omics temporal patterns.

Results: We identify individual-level responses to immune perturbation, and clusters of individuals showing similar behavior. The molecular behavior is linked to phenotypic differences, including body mass index and insulin resistance, with the immune response dominating differences attributed to diabetic status.

Conclusion: By creating appropriate networks reflecting molecular level characteristics, we capture common immune responses in individuals (healthy and pre-diabetic) in response to immune perturbation (vaccination/infection).

Funding provided by the Translational Research Institute for Space Health through NASA Cooperative Agreement NNX16AO69A (project T0412).

Abstract 3

Re-investigation of classic T cell subsets and identification of novel cell subpopulations by single-cell RNA sequencing

Xuefei Wang[1,2], Xiangru Shen[1,2], Shan Chen[1], Hongyi Liu[1], Ni Hong[1], Xi Chen[1], **Wenfei Jin1***

[1]School of Life Sciences, Southern University of Science and Technology, Shenzhen 518055, China;
[2]Equal contribution; *Corresponding author: jinwf@sustech.edu.cn (W.J.)

Classic T cell subsets are defined by a small set of cell surface markers, while single cell RNA sequencing (scRNA-seq) clusters cells using genome-wide gene expression profiles. The relationship between scRNA-seq Clustered-Populations (scCPops) and cell surface marker-defined classic T cell subsets remain unclear. Here, we interrogated 6 bead-enriched T cell subsets with 62,235 single cell transcriptomes and re-grouped them into 9 scCPops. Bead-enriched CD4 Naïve and CD8 Naïve were mainly clustered into their scCPop counterparts, while cells from the other T cell subsets were assigned to multiple scCPops including mucosal-associated invariant T cells and natural killer T cells. The multiple T cell subsets that form a single scCPop exhibited similar expression pattern, but not vice versa, indicating scCPops are much homogeneous cell populations with similar cell states. Interestingly, we discovered and named IFNhi T, a new T cell subpopulation that highly expressed Interferon Signaling Associated Genes (ISAGs). We further enriched IFNhi T by FACS sorting of BST2 for scRNA-seq analyses. IFNhi T cluster disappeared on tSNE plot after removing ISAGs, while IFNhi T cluster showed up by tSNE analyses of ISAGs alone, indicating ISAGs are the major contributor of IFNhi T cluster. BST2+ T cells and BST2- T cells showing different efficiencies of T cell activation indicates high level of ISAGs may contribute to quick immune responses.

Abstract 4

Individualized systems-level profiling of immune response using saliva

George Mias

Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

Background: Saliva offers great promise for non-invasive diagnostics (no-pain, no-needle, no-blood). This is important for monitoring vulnerable populations (e.g. the elderly or children), or where blood is not readily available: in remote locations, including underserved populations, when there are technical issues, lack of equipment or personnel, or for complex monitoring logistics (e.g. astronaut missions). In our case-study, we monitored an individual's response to pneumonia vaccination with multiomics profiling from saliva, before and after vaccination.

Methods: We sampled saliva from an individual hourly over two 24 hour periods without and with pneumococcal vaccination, and daily for 33 days spanning the vaccination response. We generated thousands of multiomics timeseries per sample from extracted saliva RNA and protein, and extracellular vesicle RNA.

Results: Over 18,000 saliva multiomics timeseries showed statistically significant temporal trends. Multiple immune response and regulation pathways were activated following vaccination, concordant with innate and adaptive immune response timeframes, coinciding with vaccination and fever reported by the subject.

Conclusion: Our results suggest that saliva omics may be used towards non-invasive personalized immune monitoring, offering the first step for saliva diagnostics with universal population utility.

Funding provided by the Translational Research Institute for Space Health through NASA Cooperative Agreement NNX16AO69A (project T0412).

Abstract 5 (No pre-recorded presentation provided)

TurboID biotin-tagging mass spectrometry identifies caspase-11-associated proteins regulating inflammasome-driven inflammation in macrophages

O. Ernst[1], C. Bradfield[1], S-H Yoon[1], A. Armstrong[2], S. Katz[1], A. Nita-Lazar[1], I. Fraser[1]

[1]Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD

[2]Bioinformatics and Computational Biosciences Branch, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD

Dysregulated activation of inflammasomes and associated pyroptosis are important drivers of inflammatory disease. While cytosolic-LPS is known to activate caspase-11, the processes regulating the non-canonical inflammasome remain poorly defined. Caspase-11 and caspase-1 show structural similarity, however no sensor or adaptor proteins are known to activate caspase-11. Also, while caspase-11 was shown to associate with LPS, it lacks the characteristic domain observed in other LPS-binding proteins. Thus, we hypothesized that other effectors may be required to activate caspase-11.

To identify novel regulators of caspase-11, we generated macrophages stably expressing caspase11-TurboID-DHFR. The destabilizing domain was included to limit pyroptosis from over-expression of caspase-11. A TurboID biotin-tagging MS-based assay was used to detect proteins in close proximity to caspase-11 +/- cytosolic LPS challenge. Importantly, this assay allowed identification of transient interactions typically missed by traditional immunoprecipitation assays. Assay hits were validated using siRNA knockdown in primary mouse macrophages.

We have identified numerous novel regulators of the macrophage response to cytosolic LPS, including proteins with pyrin and LRR domains typical of inflammasome sensors. Furthermore, several proteins interacted with caspase-11 only in the resting state, suggesting a possible role in negative regulation of this pathway, and highlighting possible targets for therapeutic targeting in inflammatory disease.

Abstract 6

Multi-block PLS integration of omics data reveals new insights into the metabolic immunomodulatory mechanisms of fasting and re-feeding

Allison Meadows1,2, Komudi Singh[3], Kim Han[1], Antonio Murgia[2], Ben McNally[2], Rebecca Huffstutler[4], Julian Griffin[2], Michael Sack[1]

[1]Laboratory of Mitochondrial Biology and Metabolism, Cardiovascular Branch, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]Department of Biochemistry, University of Cambridge, United Kingdom

[3]Bioinformatics and Computational Biology Core, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[4]Cardiovascular Branch, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Nutrient deprivation through caloric restriction or fasting has been shown to have a profound impact on reducing risk factors and symptoms of chronic disease with underlying inflammatory etiology. Studies have highlighted immune-modulatory and anti-inflammatory effects of nutrient deprivation, suggesting a critical role for metabolic and mitochondrial function on immune activity. To explore the mechanisms underlying these effects, we have carried out a clinical study to assess the immunologic effects of a 24-hour period of fasting followed by a period of re-feeding in normal volunteers. To identify the factors or metabolites involved in the nutrient-dependent regulation of immune function, we have pursued a systems modelling approach that uses transcriptomic, metabolomic, and lipidomic analysis of human PBMC samples collected through the study. Using multi-block PLS-discriminant analysis to integrate these datasets, we have validated the use of our 24-hour fasting protocol to initiate fundamental biological processes underlying the known benefits of long-term caloric restriction, and we have revealed new metabolic and transcriptional targets in nutrient-dependent immune regulation. Through functional characterization of these identified factors, we hope to elucidate the mechanisms underpinning fasting-mediated immune modulation and shed new light on the role of nutrient intake on inflammatory pathophysiology and treatment of metabolic disease.

Abstract 7

Co-Profiling of Chromatin Occupancy and RNAs in single cells

Wai Lim Ku, Lixia Pan, and Keji Zhao

Laboratory of Epigenome Biology, System Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Elucidating the relationship between cellular heterogeneity in epigenome and gene expression is critical to understand the underlying cellular states and functions. However, methods for simultaneous profiling of chromatin occupancy and RNA in the same single cell are not available currently. Therefore, we developed single-cell co-profiling of chromatin occupancy and RNAs sequencing (scPCOR-seq) for simultaneously profiling genome-wide chromatin protein binding or histone modification marks and RNA expression in the same cell. We demonstrated that scPCOR-seq is able to profile either H3K4me3 or RNAPII and RNAs in a mixture of human H1 and 293T cells at a single-cell resolution and either H3K4me3, RNAPII, or RNA profile can correctly separate the cells. We observed that the cellular variation in PolII bindings is strongly correlated with that in gene expression, which is affected by the location of polII bindings. Overall, our work provides a promising approach to understand the relationships among different omics layers.

Abstract 8

Cellular diversity in the Drosophila 3rd instar larval ventral cord revealed by single-cell transcriptomics

Tho H. Nguyen, Thomas Brody, Rosario Vicidomini and Mihaela Serpe

Section on Cellular Communication, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD

To gain insight into the cellular diversity of the larva ventral cord, we have sequenced ~30,000 single cells and assigned them to 23 clusters. We have used the expression of VGlut, Gad1, VAChT, Ddc and repo to define glutamatergic, gabaergic, cholinergic, and serotonergic/dopaminergic neurons and glia, respectively. Three clusters are identified as putative neural precursors of the adult ventral cord, based on expression of neuroblast precursor genes. We also identified two clusters as motor neurons (MNs). Within the abdominal MN population, we have identified the RP2 motor neuron, type II unpaired medial neurons, and Type III neurosecretory neurons based on their expression of known factors. A large 'supercluster' is made up over 40 interneuron types and is marked by expression of all four types of neurotransmitters, but in largely non-overlapping sets of cells. A second supercluster represents cells that constitute precursors and neural progeny that will constitute the adult CNS. Finally, we have identified eight glial clusters, including one glial precursor. Presence of multiple GPCRs and cell recognition proteins in single clusters suggest that interactions of multiple sensory circuits with single cells serve to integrate signaling inputs into single behaviors.

Abstract 9

Aberrant CTCF binding facilitates oncogenic transcriptional dysregulation in cancer

Zhenjia Wang and **Chongzhi Zang**

Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA

Background: CCCTC-binding factor (CTCF) is a transcription factor (TF) with functions in maintaining the topological structure of chromatin and inducing DNA looping. Alterations of individual CTCF binding associated with DNA methylation changes have been reported in many cell systems and result in aberrant chromatin interaction and transcriptional dysregulation.

Methods: Using an integrative computational approach, we systematically analyzed over 700 CTCF ChIP-seq datasets along with other genomics data including RNA-seq, ATAC-seq, Hi-C and Bisulfite sequencing data across different human tissues and cancer samples and identified cancer-specific patterns of gained and lost CTCF binding in six cancer types.

Results: We found that cancer-specific CTCF binding events do not always arise from changes in DNA methylation or sequence mutations. Instead, cancer-specific CTCF binding events primarily exhibit enhancer activities and are induced by oncogenic transcription factors. Gained CTCF binding may be recruited by clusters of transcription factors and co-factors in the formation of phase-separated transcriptional condensates at super-enhancers. CTCF plays an instrumental role in maintaining the active chromatin state with the transcriptional condensates, to facilitate oncogenic transcriptional activation.

Conclusion: Cancer-specific CTCF binding can be induced by other transcription factors to regulate oncogenic transcription program. Aberrant CTCF binding is an epigenomic signature of cancer.

Abstract 10 (No pre-recorded presentation provided)

Transcription factor TCF1 is indispensable for the epigenetic priming of EILPs toward distinct cell fates

Gang Ren[1], Binbin Lai[1], Harly Christelle[2], Kairong Cui[1], Avinash Bhandoola[2], and Keji Zhao[1]

[1]Laboratory of Epigenome Biology, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]Laboratory of Genome Integrity, Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD

Differentiation of Innate lymphoid cells (ILCs) from hematopoietic stem cells needs go through several progenitor stages defined by various cell surface markers. The progenitor populations at specific developmental stages may have multiple lineage potentials. However, it is not clear whether the multipotency of the progenitors are induced in situ by differentiation signals or are already predefined by epigenetic states. Here we report the profiling of genome-wide nucleosome position and chromatin accessibility simultaneously in ILC progenitors EILPs and ILCPs by single-cell MNase-Seq. We find that EILPs contain distinct sub-populations epigenetically primed toward either myeloid or ILC lineages, while ILCPs contain cells mainly primed for the ILC lineage at epigenetic level. We further demonstrate that the transcription factor TCF1 is indispensable for the epigenetic priming of lineage-defining sites (LDSs) for both myeloid lineages and ILC lineages at the EILP stage, and deletion of TCF1 results in the epigenetic priming that favors the myeloid lineages. Our results suggest that the multipotency of progenitor cells is defined by the existence of heterogeneous population of cells epigenetically primed for distinct downstream lineages, which are regulated by key transcription factors.

Abstract 11

Sensitivity of Yeast to Lithium Chloride and Regulation of Translation

Mustafa Al-gafari, Sasi Jagadeesan and Ashkan Golshani

Department of Biology, Carleton University, Ottawa, Ontario, Canada

Supervisor: Dr. Ashkan Golshani

Lithium chloride (LiCl) is a widely used and heavily studied medication used for the treatment of individuals diagnosed with bipolar disorder (BD). LiCl thus, has been under a multitude of studies examining its toxicity, action, and downstream cellular responses. LiCl is known to affect cell signaling and signaling transduction pathways through protein kinase C and glycogen synthase kinase-3, which are important aspects in regulating gene expression at the translational level. The additional downstream effects caused by LiCl require further investigation in relation to the translational pathway. In yeast, LiCl treatment is reported to reduce the activity and alters the expression of PGM2, a gene that encodes a phosphoglucomutase involved in sugar metabolism. Reduced activity of phosphoglucomutase in the presence of galactose causes an accumulation of intermediate metabolites of galactose metabolism leading to cell toxicity. In this study, we identified genes, DAN1, DAN2 and DAN3 which increase yeast LiCl sensitivity when deleted. We further demonstrate that DAN1, DAN2 and DAN3 influence translation and exert their activity through the 5'-Untranslated region (5'-UTR) of PGM2 mRNA in yeast.

Abstract 12

Single-cell RNA-seq analysis of human coronary arteries using an enhanced workflow reveals SMC transitions and candidate drug targets

Wei Feng Ma, Chani Hodonsky, Adam Turner, Doris Wong, Yipei Song, Nelson Barrientos, Jose Mosquera, and Clint Miller

Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA

Background and Aims: The atherosclerotic plaque microenvironment is highly complex, and selective agents that modulate plaque stability or other plaque phenotypes are not yet available. We sought to investigate the human atherosclerotic cellular environment using scRNA-seq to uncover potential therapeutic approaches. We aimed to make our workflow user-friendly, reproducible, and applicable to other disease-specific scRNA-seq datasets.

Methods: Here we incorporate automated cell labeling, pseudotemporal ordering, ligand-receptor evaluation, and drug-gene interaction analysis into an enhanced and reproducible scRNA-seq analysis workflow. Notably, we also developed an R Shiny based interactive web application to enable further exploration and analysis of the scRNA dataset.

Results: We applied this analysis workflow to a human coronary artery scRNA dataset and revealed distinct derivations of chondrocyte-like and fibroblast-like cells from smooth muscle cells (SMCs), and show the key changes in gene expression along their de-differentiation path. We highlighted several key ligand-receptor interactions within the atherosclerotic environment through functional expression profiling and revealed several attractive avenues for future pharmacological repurposing in precision medicine. Further, our interactive web application, PlaqView (www.plaqview.com), allows other researchers to easily explore this dataset and benchmark applicable scRNA-seq analysis tools without prior coding knowledge.

Conclusions: These results suggest novel effects of chemotherapeutics on the atherosclerotic cellular environment and provide future avenues of studies in precision medicine. This publicly available workflow will also allow for more systematic and user-friendly analysis of scRNA datasets in other disease and developmental systems. PlaqView allows for rapid visualization and analysis of atherosclerosis scRNA-seq datasets without the need of prior coding experience. Future releases of PlaqView will feature additional larger scRNA-seq and scATAC-seq atherosclerosis-related datasets, thus providing a critical resource for the field by promoting data harmonization and biological interpretation.

Abstract 13

High resolution genome architecture elucidated by Hi-TrAC

Shuai Liu[1], Yaqiang Cao[1], Qingsong Tang[1], and Keji Zhao[2]

[1]Laboratory of System Genetics, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]Laboratory of Epigenome Biology, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

The fine tuning of gene expression is regulated by chromatin looping mediated spatial contact of regulatory elements. Due to limitations of available techniques, the interaction network of regulatory elements is not well elucidated. Here we introduce High-resolution Transposase-mediated Analysis of Chromatin (Hi-TrAC) for profiling the fine architecture of chromatin. As a proximity ligation free technique, Hi-TrAC takes advantage of transposase Tn5 to bridge chromatin interacting partners. With relatively small amount of starting material (0.1 – 1 million cells) and low sequencing depth (300 million raw reads), Hi-TrAC generates a map of chromatin organization at 1 kb resolution across the genome and reveals the fine architecture at 200 bp resolution at a subset of genomic regions. By applying Hi-TrAC to different cell types, we observed cell specific active chromatin domains and enhancer-promoter loops. With more than 90 thousand detected chromatin loops, we constructed a comprehensive interaction network of regulatory elements. Hi-TrAC can serve as a powerful tool for elucidating the mechanisms of transcriptional regulation by enhancer-promoter interaction.

Abstract 14

Optimization of low cost, noninvasive RNA-sequencing to enable massive scaling of transcriptome studies

Molly Martorella[1,2], Stephane Castel[1], Alper Gokden[1], Paul Hoffman[1], Ana Vasileva[1], Tuuli Lappalainen[1,2]

[1]New York Genome Center, New York, NY

[2]Department of Systems Biology, Columbia University, New York, NY[2]Laboratory of Epigenome Biology, Systems Biology Center, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Background: Transcriptomic studies disentangle the functional nature of the genome by revealing the effects of variants on gene expression. However, the cost and invasiveness of sample collection and processing limit enrollment, and predominantly collected specimens (blood) fail to capture genetic regulatory mechanisms in tissue types central to disease processes. Optimizing low-cost, noninvasive RNA-sequencing facilitates discovery by increasing sample sizes in tissue types with greater relevancy.

Methods: Sample types include hair follicles, buccal tissue, saliva, and urine cell pellets. 304 samples were collected from 19 individuals over four time points, and samples are processed using in-house template-switching oligo and tagmentation method that reduces library prep cost by 81% compared to Illumina Truseq.

Results: We find hair follicles are a consistent and high-quality RNA sample (RIN > 7). Both hair and urine cell pellets produce robust, complex libraries with expression correlations > 0.8 across replicates. Buccal and saliva are more variable in quality. Cibersort deconvolution reveals cell types relevant to lung and kidney disease are captured. Comparisons to GTEx demonstrate hair is a suitable proxy for skin, buccal and saliva bear similarity to esophageal mucosa, whole blood, and lung, and urine samples are most similar to kidney cortex and mucosal tissues of the body. We are able to replicate known eQTLs and have similar power to detect using a subset of GTEx data. Further, we test collection of hair follicles in a clinical cohort, SPIROMICS, and find higher enrichment for lung disease signals compared to whole blood.

Conclusion: Our research findings support noninvasive RNA-sequencing as a promising approach for expanding transcriptomic studies and their potential for discovering underlying disease mechanisms.

Abstract 15 (No pre-recorded presentation provided)

Quantitative spatial proteomics reveals specific Nlrp3 and cell death mediated reprogramming of essential cellular functions and organelles

Kshiti Meera Phulphagar[1, 2], Daniel N. Itzhak[1], Stefan Ebner[1], Jonathan Swietlik[1], Georg H. H. Borner[1], Eicke Latz[2] and Felix Meissner[1]*

[1]Max Planck Institute of Biochemistry, Planegg, Germany

[2]Institute of Innate Immunity, University Hospital, Bonn, Germany

*Corresponding author

The Nlrp3 (Nod-like receptor protein 3) inflammasome is a cytosolic immune complex that assembles in response to diverse microbial or host damage derived signals leading to inflammatory cell death or pyroptosis. This pathway can also be triggered downstream of other programmed cell death routines thereby influencing their inflammatory potential. Nlrp3 activation is controlled by the cellular re-localization of its signaling components such as the recruitment of the adaptor protein Asc from the cytosol to the macromolecular inflammasome complex, and the gasdermin family member Gasdermin-D from the cytosol to the plasma membrane. Additionally, recent studies indicate that the integrity and organization of organelles such as the trans-Golgi network (TGN) and mitochondria are critical mediators of Nlrp3 activation and pyroptosis. Despite these advances, how the reprogramming of core cellular functions and compartments mediates inflammasome activation and cell death remains enigmatic.

Here we characterize the spatiotemporal subcellular rearrangements accompanying programmed cell death pathways in macrophages with a global, unbiased approach combining differential centrifugation with mass spectrometry (MS) based proteomics. Using macrophages deficient in Asc and Caspase-1, we dissect signaling events upstream and downstream of Nlrp3 inflammasome formation. We detect significant Nlrp3 associated alterations in the subcellular distribution of more than 100 proteins, including Asc and Gasdermin-D, protein complexes as well as entire organelles such as the TGN, endocytic machinery/clathrin, ribosomes and mitochondria. Comparison of this signature to different forms of cell death reveals that unique protein re-localizations include Mlkl/Ripk1/Ripk3 and Cytochrome-C/Diablo/Gasdermin-E during necroptosis and apoptosis, respectively. A common Nlrp3 activation signature characterized by the destabilization of the TGN/clathrin and/or mitochondria is however also detected during necroptosis and late apoptosis indicating substantial crosstalk of programmed cell death pathways.

Together, our approach provides a powerful discovery tool for the systematic and global spatiotemporal dissection of inflammasome activation as well as other innate immune signaling and programmed cell death programs.

Pre-Recorded Poster Presentations

Segment 2

Abstract 16

The Pyruvate-Lactate Axis Modulates Cardiac Hypertrophy and Heart Failure

Ahmad A Cluntun[1], Rachit Badolia[2], Sandra Lettlova[1], K Mark Parnell[3], Thirupura S Shankar[2], Nikolaos A Diakos[2], Kristofor A Olson[1], Iosif Taleb[2], Sean M Tatum[4], Jordan A Berg[1], Corey N Cunningham[1], Tyler Van Ry[5], Alex J Bott[1], Aspasia Thodou Krokidi[2], Sarah Fogarty[6], Sophia Skedros[2], Wojciech I Swiatek[1], Xuejing Yu[7], Bai Luo[8], Shannon Merx[3], Sutip Navankasattusas[2], James E Cox[5], Gregory S Ducker[1], William L Holland[4], Stephen H McKellar[9], Jared Rutter[10], Stavros G Drakos[11]

[1]Department of Biochemistry, University of Utah, Salt Lake City, UT
[2]Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT
[3]Vettore Biosciences, 1700 Owens Street Suite 515, San Francisco, CA
[4]Department of Nutrition and Integrative Physiology and the Diabetes and Metabolism Research Center, University of Utah, Salt Lake City, UT
[5]Department of Biochemistry, University of Utah, Salt Lake City, UT; Metabolomics, Proteomics and Mass Spectrometry Core Facility, University of Utah, Salt Lake City, UT
[6]Department of Biochemistry, University of Utah, Salt Lake City, UT; Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, UT
[7]University of Utah, School of Medicine, Salt Lake City, UT; Division of Cardiothoracic Surgery, Department of Surgery, Salt Lake City, UT
[8]Drug Discovery Core Facility, University of Utah, Salt Lake City, UT
[9]University of Utah, School of Medicine, Salt Lake City, UT; Division of Cardiothoracic Surgery, Department of Surgery, Salt Lake City, UT; U.T.A.H. (Utah Transplant Affiliated Hospitals) Cardiac Transplant Program: University of Utah Healthcare and School of Medicine, Intermountain Medical Center, Salt Lake VA (Veterans Affairs) Health Care System, Salt Lake City, UT
[10]Department of Biochemistry, University of Utah, Salt Lake City, UT; Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, UT. Electronic address: rutter@biochem.utah.edu.
[11]Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT; U.T.A.H. (Utah Transplant Affiliated Hospitals) Cardiac Transplant Program: University of Utah Healthcare and School of Medicine, Intermountain Medical Center, Salt Lake VA (Veterans Affairs) Health Care System, Salt Lake City, UT. Electronic address: stavros.drakos@hsc.utah.edu.

Background: The metabolic rewiring of cardiomyocytes is a widely accepted hallmark of heart failure (HF). These metabolic changes include a decrease in mitochondrial pyruvate oxidation and an increased export of lactate. We identify the Mitochondrial Pyruvate Carrier (MPC) and the cellular lactate exporter Monocarboxylate Transporter 4 (MCT4), as pivotal nodes in this metabolic axis.

Methods: Patients with chronic advanced HF were prospectively enrolled at the time of Left Ventricular Assist Device (LVAD) implantation. (LVAD responder patients n=12; LVAD non-responder patients n=21) Human cardiac tissue pre-LVAD was compared to post-LVAD treatment. Adult cardiac-specific MPC1-knock out mice were generated; MPC1 fl/fl; MHC-Cre-ER(T2) and compared to WT littermates.

Results: We observed that cardiac LVAD-induced myocardial recovery in chronic HF patients was coincident with increased myocardial expression of the MPC. Moreover, the genetic ablation of the MPC in cultured cardiomyocytes and in adult murine hearts was sufficient to induce hypertrophy and HF. Conversely, MPC overexpression attenuated drug-induced hypertrophy in a cell-autonomous manner. We also introduced a novel, highly potent MCT4 inhibitor that mitigated hypertrophy in cultured cardiomyocytes and in mice.

Conclusion: Together, we find that alteration of the pyruvate-lactate axis is a fundamental and early feature of cardiac hypertrophy and failure.

Abstract 17

3D development of the mitochondrial reticulum in the cardiac muscles of mice

Yuho Kim[1,4], Eric Lindberg[3], Christopher KE Bleck[3], and Brian Glancy[1,2]

[1]Muscle Energetics Laboratory, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), Bethesda, MD

[3]Electron Microscopy Core, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[4]Department of Physical Therapy and Kinesiology, University of Massachusetts Lowell, Lowell, MA

Background: Adult cardiac muscles are characterized by well-developed mitochondrial networks, which supports high energy demand in the heart. However, it is obscure how these structures are formed during postnatal development.

Methods: Using FIB-SEM, we collected high-resolution image stacks of cardiac muscles of mice at postnatal (P) day 1, 7, 14, and 42, respectively (N=2-3 per group). Then, we conducted a semi-automated machine running to extract 3D mitochondrial formations and analyzed its morphological characteristics across postnatal days.

Results: After forming more stretched structures by P7, it was shown that cardiac muscles further develop spherical shaped-mitochondria and that they are overall arranged in the fiber-parallel direction. Although total mitochondrial volumes tend to increase across P1-P42, individual mitochondrial volumes were relatively smaller during early postnatal days (P1-P14) than in the matured muscles. Notably, we observed that individual mitochondrial surface area to volume ratios as well as aspect ratios are significantly downregulated during the entire developmental period by ~34% and ~31%, respectively. Furthermore, it was also shown that individual mitochondrial lengths are ~31% decreased across the postnatal days.

Conclusion: These results provide dynamic transitions of individual mitochondrial structures during postnatal cardiac muscle development. However, to better understand functional aspects of mitochondria, it will be further needed to study intermitochondrial interactions, as well as mitochondrial interactions with other subcellular components in developing cardiac muscles.

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Abstract 18

Mitochondrial AKT Interacted with E3 Subunit of Pyruvate Dehydrogenase Complex and Modulated Myocardial Metabolism and Cardiac Function

Hank Chen and Ping Wang

Department of Diabetes, Endocrinology & Metabolism, City of Hope National Medical Center

Background: Diminished AKT translocation into mitochondria contributed to the development of diabetic cardiomyopathy.

Methods: An inducible transgenic mice expressing mitochondria-targeting dominant negative AKT (CAMDAKT) in heart. Proteomic/metabolomic analyses were performed with MALDI TOF/TOF. Interactions between AKT and Pyruvate dehydrogenase (PDH) E3 subunit were analyzed by modular dynamics simulations. Small molecule library was computationally screened for compounds that block the allosteric docking site.

Results: PDH activity was decreased in the mitochondria of CAMDKAT heart. Proteomic study revealed AKT interacted with of PDH E3 subunit. Computational screening yielded a short list of compounds that could disrupt docking of AKT-E3. In vitro pull-down assay with recombinant AKT and E3 validated two compounds with dose-responsive interruption of AKT-E3 binding. Insulin stimulation of PDH activity and formation of supercomplex in mitochondria was inhibited by the compound. This novel AKT-E3 pathway was independent of PDK. Acetyl-CoA was reduced in the CAMDAKT mitochondria. In CAMDAKT mice, cardiac mitochondria respiration was uncoupled, ATP production lower, and oxidative stress higher. LV ejection fraction was decreased.

Conclusion: We used system biology approaches and dissected the mechanism of mitochondria AKT action. Docking of AKT to PDH E3 perturbed mitochondria metabolism and led to development of heart failure.

Abstract 19

Cardiomyocyte stiffness determines the distinct morphological changes in inherited cardiomyopathy through HCM/DCM signaling network

Ali Khalilimeybodi and Jeffrey Saucerman

Cardiac Systems Pharmacology Group, Department of Biomedical Engineering, University of Virginia, Charlottesville, VA

Background: Mutations in sarcomeric proteins that cause hypertrophic (HCM) and dilated cardiomyopathy (DCM) can increase or decrease myofilament Ca2+ sensitivity, respectively. However, the cardiac signaling network mediating genotype-phenotype relationships in cardiomyopathy remains unclear.

Methods: In this study, we propose that cardiomyocyte stiffness via Titin is a hub that determines cardiomyocyte compensatory response in HCM/DCM contexts. Based on the literature, we identified major cardiac signaling modules that contribute to distinct cardiac remodeling and morphological changes in cardiomyopathy and developed an HCM/DCM signaling network model by employing a logic-based differential equation approach.

Results: The HCM/DCM model was calibrated and then validated by curated data from literature. By employing Morris sensitivity analysis, we identified interactions between Ca2+ sensitivity, twitch force, and Titin stiffness as major players of cardiomyocyte response. Structural analysis identified new potential crosstalks and by performing perturbation analysis we identified potential drug targets such as increase in both Titin stiffness & Ca2+ sensitivity, or Titin stiffness increase & mTOR activity decrease for DCM, and decrease in both Titin stiffness & mTOR activity for HCM context. Finally, we evaluated performance of existing drugs in preventing morphological changes in HCM/DCM contexts.

Conclusion: The HCM/DCM model provides a framework to design new pharmacological treatments.

Abstract 20

Classification of White Blood Cell Leukemia using RF Outperforms CNNs

William Lamberti

Department of Biomedical Engineering and the Center for Public Health Genomics, University of Virginia, Charlottesville, VA. E-mail: william.franz.lamberti@virginia.edu

Background: Acute Lumpocytic Leukemia (ALL) is a disease that affects white blood cells(WBCs) [1]. Being able to accurately classify healthy WBCs against those with ALL is crucially important. Convolutional neural networks (CNNs) are also very popular in medical imaging analysis [1]. A CNN is able to learn what features are important for a given task [2]. This is very powerful since the analyst does not need ato decide what features are required to accomplish the task. Our goal is to classify WBCs as healthy or ALL while outperforming the state of the art using 24 features using a random forest (RF). Furthermore, our approach outperforms leading approaches by about 5.20%.

Methods: We first created a complex image segmentation algorithm. The code to collect the metrics is provided at our GitHub link: https://github.com/billyl320/wbc_ luke. We collected 24 metrics and used a random forest (RF) model [3], [4], [5], [6]. The two sources of data used in this manuscript were the publically available and well regarded ALL-IDB [7] and C-NMC [8], [9], [10] datasets.

Results: Our model outperformed the other approaches by about 6.31% and 3.81% on average for the ALL-IDB2 and C-NMC datasets, respectively [1], [1], [11], [12], [13], [14], [15], [16].

Conclusion: We provide a competitive and novel solution for WBC classification of Leukemia and healthy cells. Our approach outperforms by about 5.20%.

Abstract 21

Multi-omics integrated cardiac fibroblast network modeling identifies targets of central fibroblast activation regulator MBNL1

Anders Nelson[1] and Jeffrey Saucerman[2]

[1]Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA

[2]Department of Biomedical Engineering, University of Virginia, Charlottesville, VACharlottesville, VA. E-mail: william.franz.lamberti@virginia.edu

Background: Heart failure complications following cardiac injury are acutely linked to the dysregulation of the fibrotic wound healing response. Recently, RNA-binding protein muscleblind-like1 (MBNL1) was identified as a central regulator of post-MI cardiac wound healing and myofibroblast activation in mice. Genome-wide RNA-immunoprecipitation suggests that MBNL1 regulates mRNAs in myriad influential fibroblast pathways leading to expression of -SMA, a maker of myofibroblast activation.

Methods: Our group has previously used a large-scale computational model to map signaling of regulatory linchpins for cardiac fibrosis. To identify putative MBNL1 targets, we employed a novel data mining approach combining multiple genome-wide screens with our fibroblast network model. We expanded our model to include putative MBNL1-target interactions and recapitulated published experimental results to validate new signaling modules.

Results: Through our approach, we prioritized twelve MBNL1 targets and developed novel fibroblast signaling modules for p38 MAPK, Hippo, Runx1, and Sox9 pathways. We also predicted an NFAT-SRF feedback signaling pathway regulated by p38 in cardiac fibroblasts.

Conclusion: Using the expanded fibroblast model, we predicted a hierarchy of MBNL1 regulated pathways with high influence on -SMA expression. This study lays a new foundation to study the mechanisms of MBNL1 signaling central to fibrosis regulation.

Abstract 22

Targeted Proteomics-Driven Computational Modeling of the Mouse Macrophage Toll-like Receptor Signaling Pathway

Nathan Manes[2], Jessica Calzola[3], Pauline Kaplan[2], Matthew Scandura[2], Iain Fraser[4], Ronald Germain[5], Martin Meier-Schellersheim[6], Aleksandra Nita-Lazar[1]

[1]Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD

[2]Functional Cellular Networks Section, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD

[3]Center to Reduce Cancer Health Disparities, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD

[4]Signaling Systems Section, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD

[5]Lymphocyte Biology Section, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD

[6]Computational Systems Biology Section, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD

Background: The Toll-like receptor (TLR) signaling pathway in macrophages is essential for the initiation of an effective innate immune response. Tight regulation is essential to avoid acute tissue damage and chronic inflammation.

Methods: Targeted mass spectrometry was used to measure the absolute abundance of proteins of the mouse macrophage TLR pathway. Protein-protein association and dissociation rates are being estimated using molecular dynamics.

Results: The protein abundances spanned four orders of magnitude: from 1,400 to 11 million copies per cell. Protein-protein association and dissociation rates are being estimated, and 256 association rate constants have been estimated successfully. These data are being used as parameters for Simmune pathway simulations. Phosphosite absolute quantification is being performed to produce time-course and dose-response data to be used for pathway model training, testing, and validation. Previously published proteomics, phosphoproteomics, and flow cytometry data are also being used.

Conclusion: Targeted proteomics and molecular dynamics are being used to parameterize a model of the TLR pathway. This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, NIH.

Abstract 23

A biophysically detailed computational model of the action potential of vas deferens smooth muscle

Chitaranjan Mahapatra[1,2] , Rohit Manchanda[2]

[1]CardioVascular Research Institute, University of California San Francisco, San Francisco, CA

[2]Bio Sciences & Bio Engineering Dept., Indian Institute of Technology Bombay, Mumbai, Maharashtra, India

Background: Premature ejaculation is associated with abnormal spontaneous contraction of the vas deferens smooth muscle (VDSM). The VDSM action potential (AP) generation is essential for commencing VDSM contraction. A computational VDSM model is established to explore the underlying ionic mechanisms for the AP generation.

Methods: Seven ion channels are built using ordinary differential equations in HH formalism. Then, all ion channels were integrated to generate the AP after introducing both current and neurotransmitter stimuli.

Results: The resting membrane potential is at - 50 mV. AP was simulated in the whole-cell model by applying an external stimulus current (10-30 pA), as a brief square pulse of 10 ms duration. The exhibits depolarization, repolarization and hyperpolarization phases as found in experiments. The results show that both L-type Ca2+ channel and Na+ channel play important roles in generating spike, although both L-type Ca2+ channel is the major contributor to the total inward current.

Conclusions: Our model, constrained heavily by physiological data, provides a powerful tool to investigate the ionic mechanisms underlying the genesis of VDSM electrical activity, which can further shed light on certain aspects of premature ejaculation.

Abstract 24

Using Computational Modeling Identifies the Sources of Mitochondrial ROS from the Electron Transport Chain

Quynh Duong, Yan Levitsky, Maris Dessinger, Jason Bazil

Department of Physiology, Michigan State University, East Lansing, MI

Mitochondrial reactive oxygen species (ROS) play important roles in cellular signaling; however, certain pathological conditions such as ischemia/reperfusion (I/R) injury disrupt ROS homeostasis which contributes to cell death. A major impediment to developing therapeutic measures against oxidative stress induced cellular damage is the lack of a quantitative framework to identify the specific sources and regulatory mechanisms of mitochondrial ROS production. We developed a thermodynamically consistent, mass-and-charge balanced, kinetic model of mitochondrial ROS homeostasis focusing the electron transport chain complexes I, II, and III. The model was calibrated and validated using comprehensive data sets relevant to ROS homeostasis. The model predicts that, under conditions that favor a highly reduced Q and NADH pools, sites IF and IQ produce significant amounts of ROS. The model also reveals that hydrogen peroxide production by site IF underlies the substrate-specific monotonic dependence between net ROS production and oxygen concentrations. Additionally, the model can quantify the effects of changes to mitochondrial environment on ROS production, an application that is still experimentally limited. Lastly, the model highlights the importance of furthering our understanding of the scavenging system under different conditions to establish a complete picture of mitochondrial ROS homeostasis.

Abstract 25

Comparing network complexity using random walk and memory biased random walk (MBRW) spectral dimension and multi-spectrality

Adam Craig[1], Mesut Yücel[2], Lev Muchnik[3] and Uri Hershberg[1,4]

[1]Systems Immunology Lab, Department of Immunology and Microbiology, School of Biomedical Engineering, Drexel University, Philadelphia, PA

[2]Commerzbank, London, United Kingdom

[3]Data Science Department, The Jerusalem School of Business Administrative, The Hebrew University of Jerusalem, Jerusalem, Israel

[4]Department of Human Biology, University of Haifa, Haifa, Israel

Background: Describing network complexity is a key challenge in understanding biological networks. Other authors have proposed fractal dimension and multifractality as measures of hierarchical structure and heterogeneity. We posit that a measure of network complexity must consider not only topology but the interaction of structure, time, and memory.

Methods: We calculated box-counting and cluster-growing fractal dimension, box-covering and sandbox multifractality, random-walk spectral dimension and multi-spectrality, and a novel memory-biased random walk (MBRW) spectral dimension and multi-spectrality for 12 biological networks and fully and partially randomized versions thereof. A valid measure must change in a consistent direction with increasing randomization.

Results: While sandbox multi-spectrality showed a downward trend as randomness increased, only random walk spectral dimension and MBRW multi-spectrality always changed in a consistent direction as randomness increased.

Conclusion: Random walk-based measures relate more consistently to hierarchical community structure than do topological measures of fractal dimension and multifractality. Whereas memory is not necessary for detection of the overall presence of hierarchical structure of a network, it is important to the detection of heterogeneity.

Abstract 26

Structural changes in the mitochondrial cristae network during calcium overload correlate with loss of function

Jasiel Strubbe, Benjamin West, Sundharraman Subramanian, Kristin Parent, and Jason Bazil

Department of Physiology, Michigan State University, East Lansing, MI

Myocardial ischemia/reperfusion injury is primarily driven by mitochondrial dysfunction. In this setting, mitochondrial calcium overload is thought to cause a loss of mitochondrial function and precipitate cell death. While these effects are well-known, how calcium alters the mitochondria ultrastructure and cristae network remains enigmatic. Here, we analyzed structural data using well-established cryo-electron microscopy (cryo-EM) in a population of guinea pig cardiac mitochondria exposed to high levels of calcium in the presence of the cardioprotective agent cyclosporin A (CsA). Coincident bioenergetic data were collected to explain the morphological changes induced by calcium overload. Our cryo-EM results showed that the mitochondrial cristae network is altered by high levels of calcium. These changes can be explained by the presence of calcium phosphate deposits within the mitochondrial matrix. Despite these clear morphological effects, it is unclear how structural changes in CsA-treated mitochondria due to calcium and the drug preserves function. Here, we show that the protection conferred by CsA during calcium overload is likely associated with preserved cristae density and junctional widths. Overall, these findings establish a mechanism of calcium-induced mitochondrial dysfunction and reveal new, potential targets for cardioprotective therapies responsible for maintaining cristae structure and function.

Abstract 27

Subcellular specialization of 3D mitochondrial morphology and mitochondria-lipid droplet interactivity is associated with proximity to oxygen supply in skeletal muscle cells

Thomas Willingham[1], Christopher KE Bleck[3], and Brian Glancy[1,2]

[1]Muscle Energetics Laboratory, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), Bethesda, MD

[3]Electron Microscopy Core, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Background: While morphologically distinct clusters of mitochondria have been identified within muscle cells, it remains unknown how the subcellular specialization of mitochondrial morphology and organelle interactivity support the functional demands of different subcellular regions.

Methods: Here, we combine 3D focused-ion beam scanning electron microscopy with machine learning programs to quantify individual mitochondrial structure and organelle interactivity. We evaluate over 700 individual mitochondria per cell and leverage the statistical power of this large data set to identify 3D spatial relationships among subcellular structures.

Results: We find that mitochondrial surface area to volume ratio (15.5±0.25 μm-1) is significantly correlated (R2= 0.39) to the distance to capillary, but not distance to sarcolemma (R2= 0.02). Further analysis revealed that mitochondria within close proximity (<5 μm) to capillaries (paravascular) are ~3-fold more likely to make contact with lipid droplets compared to mitochondria located in other subcellular regions. Moreover, the paravascular mitochondria in contact with lipid droplets have 75% greater volume (1.4±0.07 μm3) compared to those not connected to lipid droplets (0.8±0.03 μm3).

Conclusion: These preliminary analyses suggest that mitochondria in close proximity to the muscle oxygen supply exhibit distinct 3D morphology and organelle interactivity patterns that may function to support cellular metabolism.

Pre-Recorded Poster Presentations

Segment 3

Abstract 28

Mapping Transcriptomic Vector Fields of Single Cells

Xiaojie Qiu[1], Yan Zhang[2], Shayan Hosseinzadeh[3], Dian Yang[1], Angela Pogson[1], Li Wang[4], Matt Shurtleff[5], Ruoshi Yuan[3], Song Xu[6], Yian Ma[7], Joseph Replogle[1], Spyros Darmanis[8], Ivet Bahar[9], **Jianhua Xing**[9], Jonathan Weissman[1,10]*

[1]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA

[2]Department of Computational and Systems Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA

[3]Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA

[4]Department of Mathematics and Department of Computer Science and Engineering, University of Texas at Arlington, Arlington, TX

[5]Lycia Therapeutics, San Diego, CA

[6]Microsoft, Redmond, WA

[7]Halicioglu Data Science Institute, University of California San Diego, San Diego, CA

[8]GenenTech, San Francisco, CA

[9]Department of Computational and Systems Biology, Department of Physics, and Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA

[10]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA; Howard Hughes Medical Institute, University of California, San Francisco, CA; Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, MA.

*Corresponding Author: weissman@wi.mit.edu.

Background: Single-cell RNA-seq, together with RNA velocity and metabolic labeling, reveals cellular states and transitions at unprecedented resolution. Fully exploiting these data, however, requires dynamical models capable of predicting cell fate and unveiling the governing regulatory mechanisms.

Methods: Here, we introduce dynamo (https://github.com/aristoteleo/dynamo-release), an analytical framework that reconciles intrinsic splicing and labeling kinetics to estimate absolute RNA velocities, reconstructs continuous velocity vector fields that predict future cell fates, and finally employs differential geometry analyses to elucidate the underlying regulatory networks.

Results: We applied dynamo to a wide range of disparate biological processes including prediction of future states of differentiating hematopoietic stem cell lineages, deconvolution of glucocorticoid responses from orthogonal cell-cycle progression, characterization of regulatory networks driving zebrafish pigmentation, and identification of possible routes of resistance to SARS-CoV-2 infection.

Conclusion: Our work thus represents an important step in going from qualitative, metaphorical conceptualizations of differentiation, as exemplified by Waddington's epigenetic landscape, to quantitative and predictive theories.

Abstract 29

CAISC: A Software to Integrate Copy Number Variation and Single Nucleotide Mutations for Genetic Heterogeneity Profiling and Subclone Detection by Single-cell RNA Sequencing

Jeerthi Kannan*, **Liza Mathews***, Zhijie Wu, Neal S. Young, and Shouguo Gao**

*Both authors contributed equally to this manuscript. **Corresponding Author

Hematopoiesis and Bone Marrow Failure Laboratory, Hematology Branch, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Background: Although both copy number variations (CNVs) and single nucleotide variations (SNVs) detected by single cell RNA sequencing (scRNA-seq) are used to study intratumor heterogeneity and detect clonal groups, a software that integrates these two types of data in the same cells is unavailable.

Methods: We developed Clonal Architecture with Integration of SNV and CNV (CAISC), an R package for scRNA-seq data analysis that clusters single cells into distinct subclones by integrating CNV and SNV genotype matrices using an entropy weighted approach.

Results: The performance of CAISC was tested on simulation data and four real datasets, which confirmed its high accuracy in sub-clonal identification and assignment, including subclones which cannot be identified using one type of data alone. Furthermore, integration of SNV and CNV allowed for accurate examination of expression change between subclones, as demonstrated by the results from trisomy 8 clones of the myelodysplastic syndromes (MDS) dataset.

Conclusion: CAISC is a powerful tool for integration of CNV and SNV data from scRNA-seq to identify clonal clusters with better accuracy than obtained from a single type of data. CAISC allows a user to interactively examine clonal assignments.

Abstract 30

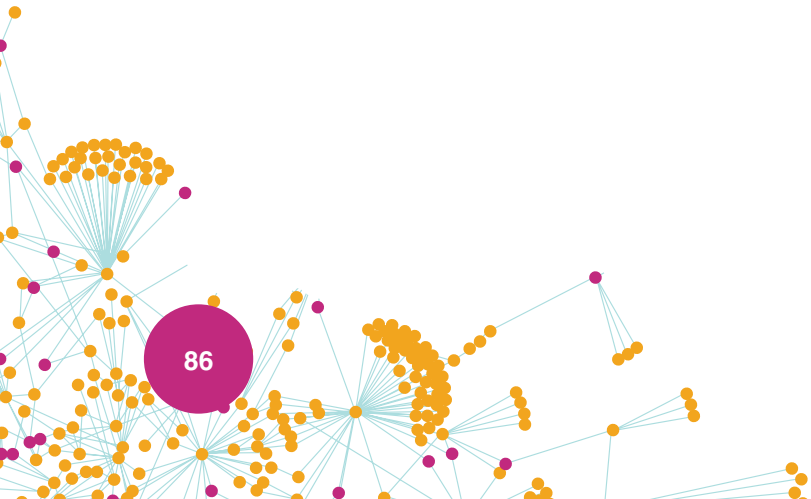
Discovery and Identification of novel genes in NHEJ (Non-Homologous End Joining) DNA Damage Pathway

Sasi Jagadeesan, Mustafa Al-gafari, and Ashkan Golshani

Supervisor: Dr. Ashkan Golshani

Department of Biology, Carleton University, Ottawa, Ontario, Canada

DNA Repair machinery defects are predominant in tumour and oncogenic cells, with mis repaired Double-stranded breaks (DSBs) posing significant challenges to cellular integrity. General DNA damage repair pathways involve Homologous Recombination (HR) or Non-Homologous End Joining (NHEJ). Continual identification of novel mechanisms involved in DSB repairs raises the expectation of additional genes still uncovered in these pathways. The DSB repair process is well maintained across eukaryotic and mammalian cells, presenting an opportunity to classify novel genes involved, via computational analysis. We developed a computational tool that performs a series of algorithms at a single instance to develop and screen for novel genes that are involved in DNA repair in Saccharomyces cerevisiae. Several databases for protein-protein interactions, genetic interactions and gene expression are screened to build an inventory of possible novel gene candidates, similar to the original gene input set of the same functionality. A screened library of candidates was computationally selected, with three novel genes shown to be involved in the DNA repair pathway – SAS1, SAS2, and SAS3. Wet-lab experimental analysis confirmed that all three novel genes SAS1, SAS2, and SAS3 are involved in the DNA damage network.



Abstract 31

Genome-wide analysis of sleep in Drosophila melanogaster subjected to constant darkness suggests context-dependent effects of genes on sleep

Surina Patel and Susan Harbison

Laboratory of Systems Genetics, Systems Biology Center, National Heart Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Environment and age affect sleep across species. Whether these changes are orchestrated by corresponding changes in the underlying genetic architecture remains unknown, however. We calculated sleep parameters from previous data measuring circadian behavior in the Drosophila Genetic Reference Panel in constant darkness (DD) for 14 days. Our goal was to compare these data to a study measuring sleep in a normal light: dark (LD) cycle for 6 days. Compared to flies in LD, flies in DD had markedly different sleep characteristics. For example, flies in DD tended to sleep less than in LD, losing 156.7 minutes of sleep on average ($P = <0.0001$). When we partitioned our data into two age groups, young (Days 1-6) and old (Days 7-13), sleep characteristics differed based on when they were measured. For example, sleep duration measured at younger ages was 31.4 minutes less than sleep measured at older ages. Associating sleep traits with 1,920,276 polymorphisms implicated 2,588 polymorphic variants. Genes associated with sleep traits differed between LD and DD flies and between young and old sleep measures, with only a 9.7% and 19.9% overlap, respectively. These results imply that the underlying genetic architecture of sleep changes depending upon both environmental condition and age.

Abstract 32

miRge3.0: A comprehensive microRNA and tRF sequencing analysis pipeline

Patil, AH and Halushka, MK*

Department of Pathology, Division of Cardiovascular Pathology, Johns Hopkins University School of Medicine, Baltimore, MD

MicroRNAs and tRNA fragments (tRFs) belong to a group of small non-coding RNAs known to regulate gene translation. Understanding the levels of their expression can help understand disease states. The advancement in next-generation sequencing has led to our ability to quantify and detect known and novel miRNAs in a high-throughput fashion. However, computational tools to efficiently handle and accurately annotate the small RNA types is a necessity. We developed miRge3.0, a robust small-RNA annotation and alignment pipeline. Python3 was employed with several packages such as concurrent. futures, pandas and cutadapt. This supported advanced parallel processing and was a more memory efficient tool than miRge and miRge2.0. miRge3.0 supports graphical user-interface developed using Electron. The features of miRge3.0 include accurate annotations of small RNAs, prediction of novel miRNAs along with advanced data quality control, processing of Unique Molecular Identifiers to account for PCR duplicates, reporting isomiRs in GFF3 format, and performing differential expression analysis. The summary provides graphical visualization with advanced interactive user controls. The performance of miRge3.0 was benchmarked to miRge2.0, Chimira and sRNAbench. We conclude our 3rd generation tool, is user friendly and supports the latest technological advancements with improvements in speed, versatility, and functionality over previous iterations.

Abstract 33

Validating polymorphisms associated with long and short sleep using Polycistronic CRISPR linked with Extreme Quantitative trait loci mapping in Drosophila melanogaster

Akanksha Singh[1], Terese Tansey[1], Carey Fagerstrom[2], Nasser Rusan[2], Susan Harbison[1]

[1]Laboratory of Systems Genetics, Systems Biology Center, National Heart Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

[2]Laboratory of Molecular Machines and Tissue Architecture, Cell and Developmental Biology Center, National Heart Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD

Sleep duration is highly variable across species. Why some organisms sleep less and some sleep more is not clear. A previous artificial selection experiment identified 126 polymorphisms putatively associated with sleep duration. Here we combined polycistronic CRISPR/Cas9 with extreme QTL mapping to verify the relevance of these polymorphisms on sleep duration. Using a polycistronic CRISPR approach we constructed two polycistronic guide RNAs targeting four distinct polymorphisms to create small genomic deletions via non-homologous end joining. Each construct was injected into flies from a long sleeping line of the Sleep Inbred Panel whose average night sleep is 697.14 +/- 2.66 min. Injected flies mated randomly for three generations. Sleep was measured in transformed populations in the F2 and F3 generations. We collected the shortest-sleeping 10% and the longest-sleeping 10% of flies from both the generations for DNA extraction.

Significantly decreased sleep was observed in both populations suggesting efficient transformation. Interestingly, the distribution of sleep in the F3 generation was narrower.

Night sleep in both populations are skewed toward short sleep suggesting transformed flies are homozygous for respective indels. Association of these phenotypes with the collected DNA will reveal the relationship between sleep and these variants.

Abstract 34

Distinguishing known from predicted with RefSeq Functional Elements, a high-confidence dataset of non-genic functional regions and interactions

Catherine M. Farrell, Terence D. Murphy, RefSeq Curation and Development Team

National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), National Institutes of Health (NIH), Bethesda, MD

Background: Eukaryotic non-genic elements function in gene regulation, chromosome organization, recombination, repair and replication. Epigenomic resources provide high coverage of predicted gene regulatory regions, but those data are difficult to access and interpret without field-specific expertise, and do not always show function when tested experimentally.

Methods: NCBI created RefSeq Functional Elements (www.ncbi.nlm.nih.gov/refseq/functionalelements/), a high-confidence dataset of experimentally validated non-genic elements in human and mouse. The dataset was curated to include richly annotated RefSeq records, descriptive Gene database records, genome annotation, and activity-supported gene regulatory and recombination interactions. Experimental evidence was derived from the literature, either focused studies, e.g. coronavirus human host gene regulatory elements, or high-throughput studies, e.g. MPRA- or CRISPRi-validated elements.

Results: The data are freely available in various formats and can be viewed in multiple genome browsers via the RefSeqFE track hub, which includes interaction tracks. The dataset provides succinct and transparent functional details, leverages data integration from multiple experimental sources, is easily accessible and adaptable, and utilizes a flexible data model, while also being complementary to epigenomic datasets.

Conclusion: The data have multiple uses in basic functional discovery, bioinformatics studies, genetic variant interpretation, as known positive controls for epigenomic studies, and as reference standards for functional interactions.

Abstract 35

Transcriptomic alternative polyadenylation signatures across human tissues shaped by functional rare genetic variants

Xudong Zou and Lei Li

Computational and Disease Genomics Laboratory, Shenzhen Bay Laboratory, Shenzhen, Guangdong, China

Background: Genome-wide association studies (GWASs) have identified thousands of common non-coding variants associated with human complex diseases. However, these studies excluded the effect of rare variants in associating with diseases. These rare non-coding variants have recently been described as associating with gene expression and splicing outliers, however interpreting these rare genetic variants still remains a significant challenge.

Methods: We identify alternative polyadenylation (APA) outliers (apaOutliers), that individuals showing extreme APA expression levels for a particular gene, across 49 human tissues from Genotype-Tissue Expression Project.

Results: We find that 37,419 transcripts were detected as single-tissue apaOutliers, and 4,247 transcripts detected as multiple-tissue apaOutliers. These apaOutliers are enriched with different functional rare variants compare to expression outliers.

Conclusions: Our findings indicate that apaOutliers explain a novel type of rare functional genetic variants and to provide new insights for interpreting individual genomes.

Abstract 36

SELMA: a computational framework for modeling intrinsic biases in chromatin accessibility sequencing data

Shengen Hu and Chongzhi Zang

Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA

Background: Genome-wide chromatin accessibility profiles measured by DNase-seq and ATAC-seq contain large noises caused by intrinsic cleavage biases. Although some existing computational tools include correction of such biases, the performance of this analysis has not been optimal due to inaccurate bias estimation.

Methods: We present Simplex Encoded Linear Model for Accessible chromatin (SELMA), a computational framework for systematic and accurate estimation of intrinsic cleavage biases to improve DNase/ATAC-seq data analysis. SELMA uses a simplex encoding model to improve bias estimation, and includes an alternative strategy for estimating biases using sequence reads mapped to mitochondria DNA. SELMA provides optimized solutions to estimate the effect of biases on genome-wide DNase/ATAC-seq cleavage sites.

Results: Using SELMA, we annotated the ENCODE consensus DNase footprint regions with estimated bias scores. We showed that transcription factor binding prediction from DNase footprint regions can be improved by incorporating the bias score. We further demonstrated improved cell type clustering of single-cell ATAC-seq data by considering the bias effect estimated by SEMLA.

Conclusion: Accurate estimation of intrinsic cleavage biases is important in chromatin accessibility sequencing data analysis. SELMA is a comprehensive computational framework for improved bias estimation, and can be a useful method in functional analysis of both bulk and single-cell DNase/ATAC-seq data.

Abstract 37

BART3D: a computational method for inferring transcriptional regulators associated with differential chromatin interactions from Hi-C data

Zhenjia Wang and Chongzhi Zang

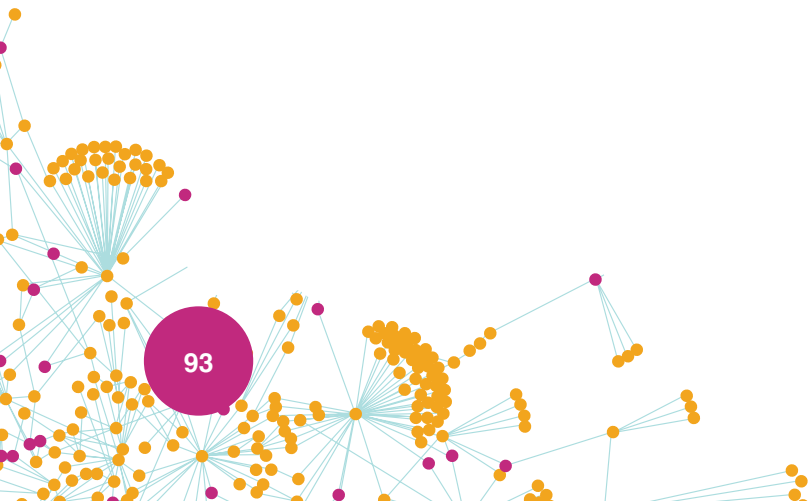
Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA

Background: Identification of functional transcriptional regulators (TRs) associated with chromatin interactions is an important problem in studies of 3-dimensional genome organization and gene regulation. It remains challenging to identify TR binding directly from low-resolution Hi-C data alone for functional analysis of 3D genome data.

Methods: We developed a computational method, BART3D, to infer TRs associated with genome-wide differential chromatin interactions by comparing Hi-C maps from two states, leveraging public ChIP-seq data for human and mouse. BART3D takes Hi-C-type 3D genome contact maps from two biological states as input, and generates a genomic differential chromatin interaction (DCI) profile by comparing the contact maps. The output of BART3D includes ranked lists of TRs associated with increased or decreased chromatin interactions with a series of statistical measurements.

Results: We use dynamic Hi-C datasets from TR KO experiments and cell differentiation to show that BART3D can infer the perturbed TRs from chromatin architecture changes and other TRs of biological relevance.

Conclusion: BART3D overcomes the relatively low resolution of Hi-C data and connects chromatin interactions on the multi-kb to Mb level to cis-regulatory events on the nucleosomal or base-pair level. BART3D can be a useful tool in 3D genome data analysis and functional genomics research.



Abstract 38

BART Cancer: a web resource for transcriptional regulators in cancer genomes

Zachary Thomas[1], Zhenjia Wang[2], Chongzhi Zang[2]

[1]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA

[2]Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA

Background: Dysregulation of gene expression plays an important role in cancer development. Identifying transcriptional regulators that drive the oncogenic gene expression program is a critical task in cancer research.

Results: Here we present BART Cancer (bartcancer.org), an interactive web resource database to display the putative transcriptional regulators that are responsible for differentially regulated genes in 15 different cancer types in The Cancer Genome Atlas (TCGA). BART Cancer also displays the activities of over 900 transcriptional regulators across cancer types, by integrating computational prediction results from BART and from the Cistrome Cancer database.

Methods: BART Cancer integrates over 10,000 gene expression profiling RNA-seq datasets from TCGA with over 7,000 ChIP-seq datasets from the Cistrome Data Browser database and the Gene Expression Omnibus (GEO). BART Cancer uses Binding Analysis for Regulation of Transcription (BART) for predicting the transcriptional regulators from the differentially expressed genes in cancer samples compared to normal samples.

Conclusion: BART Cancer is a comprehensive database focusing on transcriptional regulator activities in human cancers. BART Cancer provides unique insights into epigenetics and transcriptional gene regulation in cancer, and can be a useful resource for the broad genetics, genomics, and cancer research communities.

Abstract 39

Upregulation in the heme biosynthesis pathway increases obstructive sleep apnea severity: a mendelian randomization study

Heming Wang[1,2], Nuzulul Kurniansyah[1], Brian Cade[1,2], Han Chen[3,4], Richa Saxena[1,2,5,6], Xiaofeng Zhu[7], Alexander P. Reiner[8,9], Jerome I Rotter[10], Stephen S Rich[11], Tamar Sofer[1,2], Susan Redline[1,12], TOPMed Sleep Traits Working Group

[1]Division of Sleep and Circadian Disorders, Brigham and Women's Hospital and Harvard Medical School, Boston, MA
[2]Program in Medical and Population Genetics, Broad Institute, Cambridge, MA
[3]Human Genetics Center, Department of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX
[4]Center for Precision Health, School of Public Health and School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, TX
[5]Center for Genomic Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA
[6]Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA
[7]Department of Population and Quantitative Health Sciences, School of Medicine, Case Western Reserve University, Cleveland, OH
[8]Department of Epidemiology, University of Washington, Seattle, WA
[9]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA
[10]The Institute for Translational Genomics and Population Sciences, Department of Pediatrics, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA
[11]Center for Public Health Genomics, University of Virginia, Charlottesville, VA
[12]Division of Pulmonary, Critical Care, and Sleep Medicine, Beth Israel Deaconess Medical Center, Boston, MA

Introduction: Obstructive sleep apnea (OSA) is a common disorder associated with increased risk of cardiovascular diseases and mortality. Recent observational analyses linked genes and pathways of heme metabolism to OSA, but the causal contribution is unclear. In this study, we use gene expression data to examine whether iron and heme related pathways have a causal effect on OSA.

Methods: We performed Mendelian randomization (MR) analyses considering the expression level of 15 candidate Gene Ontology pathways as exposures and four OSA traits as outcomes. In discovery analysis, we performed two-sample MR using cis-eQTLs from the Genotype-Tissue Expression (GTEx) portal and published genome-wide summary statistics for OSA traits. Significant pathways were then followed-up by one-sample MR using high coverage DNA and RNA sequencing data from the Multi-Ethnic Study of Atherosclerosis generated by the NHLBI Trans-Omics for Precision Medicine (TOPMed) project.

Results: Discovery analysis identified putative causal associations between up-regulated heme biosynthetic process pathway on increased apnea hypopnea index and overnight hypoxemia (Pmin=0.018). These associations were supported in European and Hispanic/Latino Americans but not in African Americans in replication analysis.

Conclusion: This study suggested a causal association between heme biosynthetic processes and OSA severity, suggesting novel biomarkers and possibly treatment targets.

Abstract 40

A Meta-Analysis Exploring Age and Sex Effects on Expression Profiles for Hemorrhagic Dengue Fever

Lavida Rogers

Department of Biological Sciences, College of Science and Mathematics, University of the Virgin Islands, U.S. Virgin Islands

Background: Dengue fever (DF) is a mosquito-borne disease, caused by the dengue virus (DENV) and is prevalent in tropical/subtropical regions. DENV has 4 known serotypes and is the most prevalent infection transmitted by Aedes mosquitoes. Dengue Hemorrhagic Fever (DHF) is a fatal complication of DENV infection, with symptoms such as hemorrhaging and vascular leakage. It often occurs as a result of second DENV infections. Our study analyzes pre-existing DF expression data and defines and explores how expression profiles may be affected by sex, age and disease severity.

Methods: We are analyzing pre-existing protein array data from Brazil (12 subjects: 6 acute severe DF; 6 acute mild DF) and corresponding expression data after DF recovery. We use linear mixed effects model to investigate multiple study factors and identify differentially expressed genes, and assess disease severity, age and sex effects on DF.

Results: Our approach will highlight significant gene signatures and pathways associated with DF. Furthermore, we will identify potential gene targets for improving current treatments and exploring genes that are expressed equally across ages and sex when considering vaccinations for DENV.

Conclusion: Exploring host systemic responses can help characterize and differentiate between genetic expression profiles for non-severe and severe DF.

Abstract 41

Meta-analysis of gene expression variability in asthma

Ellaina Wyllis and Lavida Rogers

Department of Biological Sciences, College of Science and Mathematics, University of the Virgin Islands, U.S. Virgin Islands

Background: Asthma is a long-term inflammatory disease of the airway producing symptoms such as wheezing, coughing, chest tightness, and breathlessness. The Centers for Disease Control and Prevention reported chronic lower respiratory diseases, such as asthma, as the 4th leading cause of death in the United States. The underlying cause of asthma is still not fully understood.

Methods: We conducted a meta-analysis of publicly available microarray data from asthmatic and healthy individuals. We curated datasets that reported the subject's sex and disease state (4 datasets, 800 samples). We pre-processed the raw microarray expression data in R and conducted an analysis of variance that incorporated sex and disease state as effects.

Results: We identified 1,641 differentially expressed genes for disease state ($p<0.05$). However, we did not find any interactions between disease state and sex for these genes. Interesting Reactome pathways identified as enriched include neutrophil degranulation and mitochondrial calcium ion transport. Enriched gene ontology terms include hematopoietic cell lineage and prostate cancer.

Conclusion: With our current findings and future analyses, we aim to highlight potential gene and pathway associations that can hopefully be targeted to develop personalized treatment and preventative care for asthmatics.

NHLBI Office of
the Scientific Director
Division of Intramural Research
National Heart, Lung, and Blood Institute
Building 10CRC, Room 4-1581
10 Center Drive MSC 1458
Bethesda, MD 20892

National Heart, Lung,
and Blood Institute